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Review

In Vitro Human Haematopoietic Stem Cell Expansion and Differentiation

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Abstract: The haematopoietic system plays an essential role in our health and survival. It is comprised of a range of mature blood and immune cell types, including oxygen-carrying erythrocytes, platelet-producing megakaryocytes and infection-fighting myeloid and lymphoid cells. Self-renewing multipotent haematopoietic stem cells (HSCs) and a range of intermediate haematopoietic progenitor cell types differentiate into these mature cell types to continuously support haematopoietic system homeostasis throughout life. This process of haematopoiesis is tightly regulated in vivo and primarily takes place in the bone marrow. Over the years, a range of in vitro culture systems have been developed, either to expand haematopoietic stem and progenitor cells or to differentiate them into the various haematopoietic lineages, based on the use of recombinant cytokines, co-culture systems and/or small molecules. These approaches provide important tractable models to study human haematopoiesis in vitro. Additionally, haematopoietic cell culture systems are being developed and clinical tested as a source of cell products for transplantation and transfusion medicine. This review discusses the in vitro culture protocols for human HSC expansion and differentiation, and summarises the key factors involved in these biological processes.

Keywords: haematopoietic stem cells; haematopoiesis; erythrocyte; megakaryocyte; neutrophil; monocyte; B cell; T cell; self-renewal; differentiation; expansion; in vitro

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1. Introduction

The haematopoietic system plays essential roles in human health and survival. Erythrocytes oxygenate our tissues and platelets (from megakaryocytes) prevent bleeding, while myeloid cells (including neutrophils and monocytes) and lymphoid cells (including B and T cells) fight against infections. A continuous production of new blood cells is required to maintain haematopoietic system homeostasis, a process known as haematopoiesis. Self-renewing multipotent haematopoietic stem cells (HSCs) and a range of intermediate haematopoietic progenitor cell (HPC) types sustain haematopoiesis throughout life. In the adult, haematopoietic stem and progenitor cells (HSPCs) largely reside in the bone marrow (BM), in a specialised microenvironment or "niche" where HSC maintenance, self-renewal and differentiation are strictly regulated [1–3]. Adult HSPCs can also be mobilised into peripheral blood (mPB). During development, HSPCs are primarily found in the fetal liver, and can also be collected from umbilical cord blood (UCB). Throughout life, most HSPCs are marked by the surface marker CD34, which is often used for enrichment.

A large research effort has gone into identifying and characterising HSCs. HSCs are functionally defined by their ability to stably reconstitute the entire blood and immune systems following transplantation. HSCs can be prospectively isolated using fluorescence activated cell sorting (FACS) based on surface marker expression. In humans, HSCs are commonly immunophenotypically defined as $CD34^+CD38^-CD49f^+CD90^+CD45RA^-lineage^-$ cells [4]. However, immunophenotypic HSCs display heterogeneous self-renewal and differentiation potentials. Functional long-term HSCs (LT-HSCs) support life-long blood

production and are commonly quiescent, which is thought to help protect them from detrimental stress and maintains their capacity for self-renewal [2]. In the classical model of haematopoiesis, LT-HSCs can give rise to short-term HSCs (ST-HSCs), which have a limited self-renewal potential and can differentiate into multipotent progenitors (MPPs). MPPs can differentiate into lineage-committed progenitors such as those that give rise to myeloid cells (common myeloid progenitors; CMPs) and to lymphoid cells (common lymphoid progenitors; CLPs). Lineage-committed progenitors have a limited self-renewal capacity and differentiation potential, and give rise to specific mature blood and immune cell types [5].

Over the years, the development of in vitro models of HSPC differentiation has been critical for deciphering the mechanisms regulating normal and disease haematopoiesis. Additionally, there is growing interest in the use of in vitro expanded and/or generated blood cells for clinical transfusion and HSC transplantation (HSCT) therapies. Here, we summarise the key methods and regulatory factors for in vitro human HSPC expansion and their differentiation into erythrocytes, megakaryocytes, myeloid cells and lymphoid cells (Figure 1).

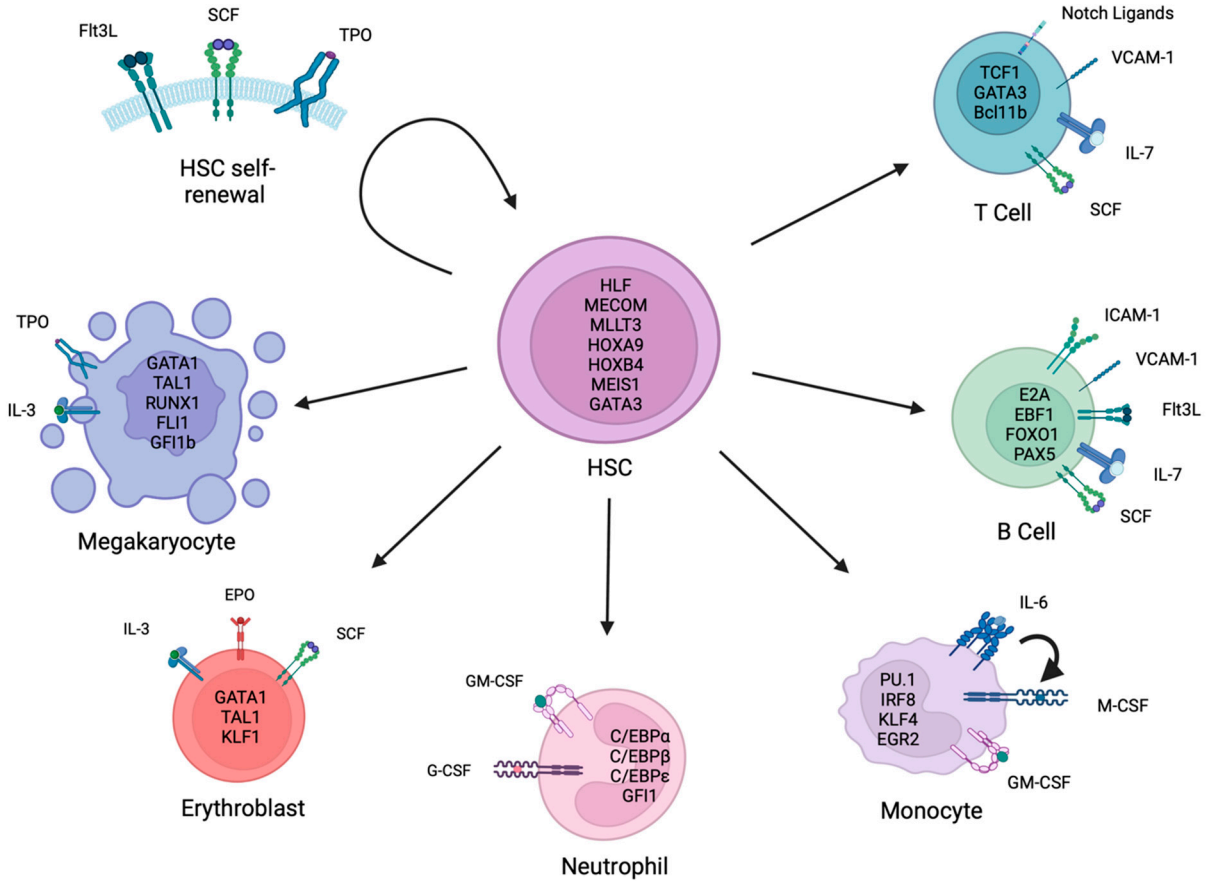

Figure 1. Key factors and pathways involved in haematopoietic stem cell self-renewal and differentiation. Haematopoietic stem cells (HSCs) can either self-renew to generate more HSCs or differentiate into the megakaryocyte, erythroid, myeloid (including neutrophil and monocyte) or lymphoid cell (including B cell and T cell) lineages. The key cytokine signalling pathways and transcription factors that regulate HSC self-renewal and differentiation are summarised. This figure was created using www.Biorender.com.

2. In Vitro Maintenance and Expansion of HSPCs

A wide range of approaches have been tested in efforts to expand HSCs in vitro (Figure 2). HSC expansion protocols have powerful uses in basic research, but also have direct translational applications to help boost donor HSC numbers for HSCT therapies.

In vitro HSC culture conditions are also necessary for ex vivo HSC gene therapies. However, stable HSC expansion in vitro remains a major challenge. The current gold standard assay for human HSC activity is the ability for serial engraftment in immunodeficient NOD-SCID or NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice. These cells are referred to as NOD-SCID repopulating cells (SRCs) [6] and their absolute number is quantified by limiting dilution assays (LDAs) [7].

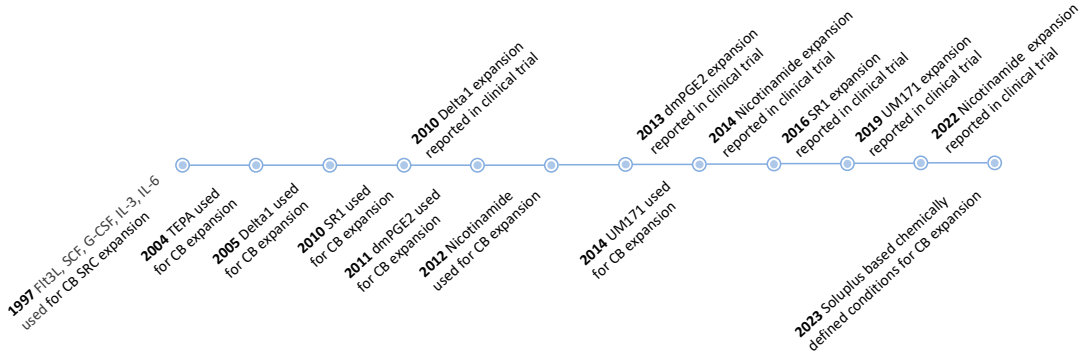

Figure 2. Timeline of culture conditions for improving HSC expansion. This figure was created using Microsoft PowerPoint.

2.1. Signalling Pathways

Extracellular cues and intracellular factors regulate HSC cell-fate decisions and cell cycle entry and exit. Many conserved pathways form the intricate signalling network that regulates HSC cell fate to achieve the delicate balance between HSC self-renewal and differentiation that ensures life-long haematopoiesis. Downstream of the conserved signalling mechanisms sits a network of transcription factors (TFs) that regulate the expression of genes that participate in processes that maintain HSC self-renewal and potential. The complete list of HSC TFs is yet be elucidated, but a number of key regulators have been identified, including MECOM, MLLT3 and HOX genes (including HOXA9 and HOXB4), HLF, GATA3 and MEIS1 [8–15]. There are several signalling pathways that play an important role in regulating HSC cell-fate decisions, including Wnt and Notch signalling [16–21]. Other signalling pathways also play significant roles in HSC regulation, including the TGF-β/Smad, JAK/STAT and PI3K/AKT pathways [20,22]. These conserved signalling pathways are activated by a number of extrinsic factors, including small, secreted signalling proteins called cytokines.

Various cytokines have been used for in vitro maintenance and expansion of human HSPCs capable of repopulating immunodeficient mice, starting from the late 1990s [23,24]. The most widely used combination includes stem cell factor (SCF), thrombopoietin (THPO), Fms-like tyrosine kinase 3 ligand (FLT-3L) and interleukin-6 (IL-6) [25], which are discussed below. However, these basic expansion culture systems are incapable of stably maintaining HSC self-renewal, which leads to depletion of HSC activity and an expansion of HPCs in the cultures. As discussed below, combinations of these cytokines are also used in HSPC differentiation protocols. Other cytokines implicated in HSC maintenance include pleiotrophin (PTN), the angiopoietin-like proteins (Angptl) and sonic hedgehog (Shh). PTN is a heparin-binding growth factor secreted by cells in the BM niche, and it regulates HSC self-renewal via inhibition of protein tyrosine phosphatase-ζ (PTPζ) [26–29]. The addition of PTN promotes expansion of human UCB HSCs [29]. The Angptl protein family plays roles in lipid metabolism, angiogenesis, inflammation, cancer cell motility and HSC expansion [30]. The addition of soluble Shh, a Hedgehog family protein, has been shown to induce human CD34$^+$CD38$^-$ cell proliferation and increase HSC repopulating ability by inhibiting bone morphogenetic protein (BMP) signalling [31]. While most HSPC expansion cultures are undertaken in traditional tissue culture plates, the novel use of 3-dimensional

zwitterionic hydrogels has recently been shown to substantially improve cytokine-mediated HSC expansion [32].

SCF: SCF was first identified as the ligand for c-Kit (CD117) in mice [33] and cloned in 1990, where it was shown to promote the expansion of haematopoietic progenitor cells [34,35]. SCF was later shown to stimulate the survival and proliferation of HSCs [36,37]. SCF is expressed by cells in the BM niche to promote HSC maintenance, and it stimulates cell cycle entry by activating the PI3K/AKT/FOXO pathway [38–40]. Signalling downstream of SCF is reviewed extensively elsewhere [41].

THPO: THPO and its receptor MPL were discovered and cloned in 1994. THPO was first shown to promote the development and maturation of megakaryocytes [42–47]. However, THPO is also required for HSC maintenance and expansion after transplantation [48–50] through its ability to induce self-renewing cell division [51], and loss of THPO signalling causes a decrease in HSC numbers [52]. THPO signalling via the MPL receptor-ligand complex results in the activation of multiple signal transduction pathways, including Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) [53–56].

FLT3L: FLT3L was first cloned in 1993 and was found to stimulate the growth of HSPCs [57,58]. It also plays a key role in lymphoid differentiation, which is discussed in the lymphoid differentiation section below. FLT3L binds and activates the receptor tyrosine kinase (RTK) Fms-like tyrosine kinase 3 (FLT3) [59]. Activation of FLT3 by FLT3L activates the downstream PI3K, MAPK/ERK and JAK/STAT3/5 pathways, and also induces Src kinase activity [59]. Constitutive activation of FLT3L signalling drives proliferation of undifferentiated myeloid progenitors [60,61]. This is mirrored in vitro, where FLT3L promotes expansion of myeloid progenitors without differentiation to mature cells, in synergy with IL-3, GM-CSF, G-CSF and M-CSF [62,63]. Signalling downstream of FLT3 is reviewed in detail elsewhere [59].

IL-6: IL-6 is a pleiotropic cytokine which modulates expansion of multiple cell types, including HSPCs, B cells, T cells and monocytes [64]. IL-6 signalling is mediated by two different pathways: classic and trans-signalling. In classical signalling, IL-6 binds to a secreted form of the IL-6 receptor (IL-6R), whereas in *trans* signalling it binds membrane-bound IL-6R. In both cases, ligand bound IL-6R subsequently interacts with GP130, which facilitates recruitment of JAK1/JAK2/TYK2 and SRC kinases [65–67]. The activated JAK proteins phosphorylate tyrosine residues of GP130, which act as docking sites for proteins that initiate PI3K/AKT, RAS/RAF/MEF/MAPK and JAK/STAT1/3/5 signalling [64,65]. IL-6 signalling is reviewed in detail elsewhere [64,65].

Notch ligands: Notch signalling regulates various processes in HSPCs in a context-specific manner [19] and several Notch ligands have been shown to maintain HSC function. Jagged1, a Notch ligand, enables a modest HSC expansion in vitro and enhances long-term engraftment in immunodeficient NSG mice [68,69]. Additionally, expression of human Jagged1 in a transgenic hJ1-NOG mouse model induced a drastic increase in the expansion of transplanted human UCB HSCs [70]. Delta1, another Notch ligand, was used in combination with cytokines for in vitro expansion of human $CD34^+CD38^-$ UCB HSPCs. This showed an over 100-fold increase in HSPC numbers, higher SRC frequency and enhanced short-term repopulating activity in a low Delta1 dose culture [71–73]. A phase I clinical trial was performed using Delta1-expanded combined with non-expanded UCB HSCs and demonstrated that the expanded fraction exhibited rapid myeloid reconstitution, but only short-term repopulating activity [72].

Co-culture systems. Mimicking the BM niche has also been explored using co-culture expansions system that employ mesenchymal stromal cells (MSCs). In a phase I clinical trial (NCT00498316) UCB $CD34^+$ cells were cultured on STRO-3^+ MSCs supplemented with SCF, THPO, FLT3L and G-CSF [74]. A 14-day expansion increased $CD34^+$ cell numbers by 30.1-fold and reduced time for haematopoietic recovery, but showed an impaired engraftment ability. More recently, a 3D MSC culture system composed of multicellular

spheroids embedded in extracellular matrix has been shown to mimic the functionality of BM niche cells, maintain the expression of stem cell markers and release haematopoietic maintenance factors [75,76]. These approaches show promise as an in vitro tool to study the microenvironment in which HSCs reside.

2.2. Small-Molecules

Due to the limited success of in vitro HSC expansion using recombinant cytokines, the field has turned to implementing small-molecule approaches to improve in vitro HSC expansion, with several now tested in HSCT clinical trials. These small molecules are often used in combination with recombinant cytokines. This has yielded a large selection of new druggable pathways implicated in HSC maintenance and expansion, as summarised below and in Table 1.

Tetraethylenepentamine (TEPA): Expansion of UCB-derived $CD34^+$ HSPCs with TEPA resulted in HSPC expansion and enhanced repopulating capacity in immunodeficient NSG mice [77,78]. TEPA is a high affinity copper chelator which was used to demonstrate that low copper conditions inhibited differentiation of UCB-derived HSPCs and promoted their proliferation [79,80]. The first phase I/II clinical trial to evaluate TEPA expanded UCB HSCs reported limited cell expansion and slow neutrophil and platelet recovery, but had high overall survival [81]. However, a second clinical trial reported higher cell expansion rates, faster haematopoietic recovery and had survival rates similar to the control group [82].

StemRegenin-1 (SR1): SR1 was identified from a small-molecule screen of a 100,000-compound library designed to detect compounds capable of promoting UCB-derived HSPC expansion. In combination with cytokines, the addition of SR1 led to a 50-fold expansion of UCB $CD34^+$ HSPCs and a 17-fold increase in SRC numbers [6]. SR1 is a purine derivative which inhibits the aryl hydrocarbon receptor (AHR) in human HSPCs. Phase I/II clinical study with SR1-expanded UCB units has reported significant $CD34^+$ HSPC expansion, enhanced engraftment and rapid haematopoietic recovery [83]. However, they observed a high incidence of transplant-related mortality and low overall survival. Two phase II trials (NCT03406962 and NCT03674411) are currently evaluating the use of SR1-expanded UCB cells for the treatment of haematologic malignancies and inherited metabolic disorders.

Nicotinamide (NAM): NAM was found to enhance UCB-derived $CD34^+$ HSPC expansion 80-fold, and transplantation assays showed an increase in SRCs and improved engraftment potential by enhancing CXCR4-CXCL12-based homing. NAM is a form of vitamin B3 which selectively inhibits the histone deacetylase Sirtuin1 (SIRT1), and inhibition of SIRT1 using the selective inhibitor EX-527 was found to mimic NAMs effect on HSPC expansion [84]. Two phase I/II clinical studies with NAM-expanded UCB units have showed a low incidence of long-term engraftment failure after expansion and high survival rates [85,86]. A phase III trial (NCT02730299) on patients with haematologic malignancies is ongoing.

Prostaglandin E2 (PGE2): PGE2 is a potent inflammatory mediator that is generated by cyclooxygenase 2 (COX2) and it can regulate the stability of β-catenin via cAMP/PKA signalling to activate the Wnt pathway [87,88]. A stable PGE2 derivative, 16,16-dimethyl-PGE2 (dmPGE2), was shown to increase CFU activity and SRC frequency in mice [89]. Expansion of UCB with dmPGE2 improves HSC self-renewal and enhances engraftment in NSG mice [90]. A phase I clinical trial using dmPEG2 showed a rapid haematopoietic recovery and higher long-term engraftment from UCB units treated with dmPEG2 [91]. Modulating endogenous PGE2 levels is also an attractive method for improving HSCT efficacy. One compound, SW033291, was identified in a 230,000 small-molecule screen. SW033291 inhibits prostaglandin-degrading enzyme 15-PGDH and raises PGE2 levels in the BM [92]. SW209415, a second generation 15-PGDH inhibitor, was shown to enhance human HSC homing and engraftment in xenograft transplants [93].

UM729 and UM171: UM729 was identified from a small-molecule screen of a 5280-compound library designed to detect compounds capable of promoting expansion of human $CD34^+$ HSPCs [94]. Structure-activity relationship (SAR) optimization of over

300 UM729 analogues yielded UM171 as the top candidate with more than 10-fold higher HSPC expansion activity. Both UM729 and UM171 are pyrimidoindole derivatives whose complete mechanism of action is not understood, but are thought to act to repress genes involved in erythroid and megakaryocytic differentiation through degradation of the epigenetic regulator lysine-specific histone demethylase 1A (LSD1) [94,95]. Transplantation assays showed that expansion with UM171 enhances LT-HSC frequency over 13-fold and maintains long-term repopulating capacity [94]. A phase I/II clinical study with UM171-expanded UCB units showed no graft failure after expansion and high survival rates [96]. Three more clinical trials (NCT03441958, NCT03913026 and NCT04103879) are under way to further evaluate the safety and efficacy of UM171 expanded HSCs in treating haematologic malignancies. The synthesis of novel pyrimidoindole analogues for HSC expansion remains an attractive approach and there are more pyrimidoindole compounds pending evaluation [97].

THPO Mimetics: Thrombopoietin (THPO) signalling through its receptor c-MPL plays an important role in HSC self-renewal. The small-molecule c-MPL-agonist NR101 induces activation of STAT5 and accumulation of HIF-1α to increase expansion of $CD34^+$ HSPCs and total SRC numbers in culture [98]. Eltrombopag, a small molecule activator of c-MPL, can affect haematopoiesis in a THPO-independent pathway through its iron chelating activity. Eltrombopag reduces iron concentration in HSPCs, which in turn decreases cellular reactive oxygen species (ROS) levels and promotes human HSPC self-renewal in vitro [99,100].

MAPK Inhibition: MAPKs regulate a wide variety of cellular processes, including cell growth, migration, proliferation, differentiation and survival. The three major MAPKs–extracellular-signal-regulated kinases (ERKs), Jun amino-terminal kinases (JNKs), and stress-activated protein kinases (p38MAPKs) can be regulated by cytokines and growth factors that play crucial roles in haematopoiesis [101]. There have been a number of studies demonstrating that targeting MAPKs may be a promising strategy for promoting HSPC expansion. Inhibition of p38 by SB203580 promotes the expansion, self-renewal and repopulating capacities of UCB HSPCs [102]. SAR studies have yielded an analogue of SB203580 called C7, which showed enhanced HSC expansion activity and promoted long-term repopulation of UCB-derived HSCs in primary and secondary NSG mice recipients [103]. JNK inhibition by JNK-IN-8 can expand human UCB HSPCs, increase SRC frequency 3.88-fold in primary recipients and has demonstrated engraftment in secondary recipients [104,105].

P18 Inhibition: The cyclin-dependent-kinase (CDK) inhibitor p18 regulates the cell cycle by inhibition of the CDK4/6 signalling pathway. Absence of p18 promotes self-renewal and expansion of HSCs in vitro [106–110]. Potent p18 inhibitors P18IN011 and P18IN003 were identified from an in silico screen and could support in vitro expansion of mouse HSCs with improved engraftment capacity in serial transplants [108]. Another p18 inhibitor that was developed in silico, called 005A, can promote robust in vitro expansion of human UCB-derived HSPCs and enhance repopulating capacity [110].

Histone Deacetylase (HDAC) Inhibition: Epigenetic modifications have a major role in cell-fate decisions and the proteins that are involved in their establishment, maintenance and removal are an appealing target for pharmacological intervention. Valproic acid (VPA) is a non-specific HDAC inhibitor that has been commonly used for the treatment of a number of neurological disorders. VPA has been shown to promote HSPC proliferation and maintenance, and it upregulates HSC self-renewal genes such as *HOXB4* [111,112]. There is evidence to suggest that HSPC expansion induced by VPA is accompanied by cellular reprogramming [113–115]. VPA treatment promotes HSC multi-lineage engraftment in serial transplantation of expanded UCB-derived HSPCs in immunodeficient NSG mice [115,116].

Histone Acetyltransferase (HAT) Inhibition: Garcinol and isogarcinol are non-selective HAT inhibitors identified in a natural compound screen for HSC expansion activity [117]. Garcinol facilitated a 4.5-fold expansion of human UCB-derived HSC and increased the expression of a key HSC factor, *HLF*, which was accompanied with a doubling of SRC numbers in immunodeficient NSG mice.

DNA Methyltransferase Inhibition: Combined treatment with 5-aza-2′-deoxycytidine (5azaD), a DNA methyltransferase inhibitor, and trichostatin A (TSA), a HDAC inhibitor, enhances UCB HSPC expansion and engraftment potential in immunodeficient NSG mice [118]. There is evidence to suggest that the combination of 5azaD and TSA preserved HSC potential by reducing the rate of cell division, whilst promoting stem cell maintenance as genes involved in self-renewal, including *HOXB4*, were upregulated alongside cell cycle inhibitors *p21* and *p27* [119,120].

BET inhibition: CPI-203 was identified in a small HSPC expansion and engraftment screen for small-molecules targeting bromodomain-containing proteins (BCPs). BCPs take part in the regulation of gene expression by recognising specific epigenetic modifications. CPI-203 inhibits BCPs that harbour a specific bromodomain and extra-terminal motif (BET) domain. Short treatment with CPI-203 promotes UCB expansion and improves long-term repopulating capacity in vivo; however, it also promotes megakaryocyte expansion [121].

Glycogen synthase kinase-3 (GSK-3) inhibition: GSK-3 plays a major role in many signalling pathways critical for HSC fate determination such as Wnt, Notch and Hedgehog signalling. Treatment with GSK-3 inhibitor CHIR99021 enhanced haematopoietic recovery in immunodeficient NSG mice [122]. In combination with insulin, it inhibits differentiation and promotes self-renewal, leading to an increase in the number of engraftable HSCs [123]. UCB-derived HSPC expansion with another GSK-3 inhibitor, BIO, prolonged the cell cycle by upregulating the CDK inhibitor p57 and down-regulating cyclin D1. However, this resulted in an increased total number of HSCs and maintained the frequency of repopulating cells in vitro by promoting Notch and Angpt1/Tie2 signalling [124]. CHIR99021 has also been used in combination with the mTOR inhibitor rapamycin to promote HSC self-renewal and enhance engraftment in NSG mice [125].

Very recently, a serum albumin and cytokine-free HSC expansion system was described, which relies on the co-polymer Soluplus (or another polymer, polyvinyl alcohol) and solely employs small-molecules to stimulate HSC maintenance signalling. A PI3K activator (740 Y-P), a THPO-receptor agonist (Butyzamide) and the pyrimidoindole derivative UM171 were used to support 30-day expansion of UCB HSCs that are capable of serial engraftment in xenograft transplantation assays [126]. This culture system is a major step toward the development of chemically-defined HSC culture systems capable of long-term expansion coupled with stem cell maintenance.

Table 1. Summary of small molecules used to improve human HSPC expansion.

Compound	Activity	Expansion Effect on HSPC Culture	Refs.
TEPA	Copper chelator	30.5-fold $CD34^+CD38^-$ HSPCs 172-fold CFU activity Improved engraftment in xenograft transplants	[78]
		219-fold total nucleated cells 6-fold $CD34^+$ HSPCs 37.8-fold CFU activity	[81]
		400-fold total nucleated cells 77-fold $CD34^+$ HSPCs	[82]
SR1	AHR	50-fold $CD34^+$ HSPCs 17-fold SRCs	[6]
		854-fold total nucleated cells 330-fold $CD34^+$ HSPCs	[83]
NAM	SIRT1	80-fold $CD34^+$ HSPCs 9-fold SRCs	[84]
		486-fold total nucleated cells 72-fold $CD34^+$ HSPCs	[85]
		33-fold $CD34^+$ HSPCs	[86]

Table 1. *Cont.*

Compound	Activity	Expansion Effect on HSPC Culture	Refs.
dmPGE2	15-PGDH	1.4-fold CFU activity Improved engraftment in xenograft transplants 2.2-fold increase in BM homing	[90,91]
UM171	LSD1	13.4-fold SRCs	[94]
		35.4-fold $CD34^+$ HSPCs Improved engraftment in xenograft transplants	[96]
NR101	THPO	4.9-fold $CD34^+CD38^-$ HSPCs 2.9-fold SRCs	[98]
Eltrombopag	THPO/Iron Chelator	1.42-fold $CD34^+CD38^-$ HSPCs	[99]
C7	p38	1554-fold $CD34^+CD38^-CD45^+CD45RA^-$ HSPCs 2.5-fold SRCs	[103]
JNK-IN-8	JNK	8-fold $CD34^+CD38^-CD45RA^-CD90^+$ HSPCs 3.88-fold SRCs	[104]
005A	p18	2.72-fold CFU activity Improved engraftment in xenograft transplants	[110]
VPA	HDAC	194.7-fold CD34+CD45+ HSPCs 6-fold SRCs	[116]
VPA	HDAC	213-fold CD34+ HSPCs 36-fold SRCs	[115]
Garcinol/ isogarcinol	HATs	4.5-/7.4-fold $CD34^+CD38^-$ HSPCs 2.5-fold SRCs (garcinol)	[117]
5azaD	DNMT	12.5-fold $CD34^+CD90^+$ HSPCs 9.6-fold SRCs	[118]
TSA	HDAC		
CPI-203	BET	5–10-fold $Lin^-CD34^+CD38^-CD45RA^-CD90^+CD49f^+$ HSPCs 1.5–3-fold SRCs	[121]
BIO	GSK-3	2-fold CFU activity	[124]
CHIR99021	GSK-3	7-fold total nucleated cells 5-fold SRCs	[125]
Rapamycin	mTOR		

3. In Vitro Differentiation to Megakaryocytes

Platelet-producing megakaryocytes are a rare blood cell type representing only 0.01% of all nucleated cells in the BM [127], and this makes them difficult to isolate and culture for research and for the production of platelets for clinical purposes. Platelets play major roles in haemostasis, thrombosis, inflammation, vessel constriction and repair, but only have a lifespan of up to 10 days, and need to be produced constantly by the body [128,129]. Failure to maintain platelet levels results in thrombocytopenia. Thrombocytopenia is commonly seen in haematological diseases, including haematopoietic aplastic anaemia, leukaemia and BM abnormalities, but may also arise from infectious, connective tissue and liver diseases, as well as chemotherapy/radiotherapy [128,130,131]. Platelet transfusion is an effective treatment that reduces the mortality caused by bleeding in these conditions. Platelets currently used in the clinic are solely donor-derived, and with the development of new clinical treatment options, the demand for platelet transfusion is increasing. This has led to an unmet need, and research has been focused on alternative strategies to obtain platelets, including in vitro platelet production.

Megakaryocytes are generated from HSCs through a stepwise process of differentiation [132,133]. A number of megakaryocyte differentiation protocols have been developed (Table 2), which commonly employ the platelet markers glycoprotein IIb/IIIa (CD41) and

glycoprotein Ib (CD42b), and/or measure cell ploidy to assess culture purity. However, current protocols for producing megakaryocytes from HSCs suffer from low differentiation efficiency, inconsistent in vivo functional testing and difficulty in scaling up production due to high costs. One of the earliest attempts to create platelets in vitro from $CD34^+$ PB HSPCs used human and aplastic canine serum to induce megakaryocyte differentiation with a very low differentiation efficiency and low platelet generation [134]. Later, differentiation efficiency was improved via the use of cytokines, namely THPO, either alone or in various combinations with SCF, IL-3 and IL-6. This demonstrated that the presence of THPO had the strongest effect on accelerating megakaryocyte differentiation [135]. Interestingly, elevated temperature has been shown to have a positive effect on differentiation of HSCs to megakaryocytes. Maintaining the culture at 39 °C promoted proliferation and megakaryocyte differentiation efficiency, and improved platelet output [136]. Large-scale differentiation processes using UCB-derived HSPCs and co-culture with telomerase-expressing human stromal cells and a three-phase cytokine cocktail system have produced larger numbers of platelets that were morphologically and functionally similar to those from plasma [137]. More recently, clinical-grade protocols for megakaryocyte generation from UCB-derived HSPCs have been developed using human albumin and a defined cocktail of cytokines and supplements [138]. Finally, 3D culture methods (roller-bottle cell culture system) have been able to further improve the efficiency of megakaryocyte and platelet generation from UCB-derived HSPCs [139,140].

Table 2. Summary of protocols for in vitro megakaryocyte differentiation.

Cell Source	Method	Cells Generated	Reference
PB $CD34^+$	human/canine serum	>95% $CD41^+$ megakaryocytes	[134]
PB $CD34^+$	THPO	>79% $CD41^+$ megakaryocytes	[135]
UCB $CD34^+$	THPO, SCF, IL-6, FLT3L	>80% $CD41^+$, >50% $CD41^+CD42b^+$ megakaryocytes	[136]
UCB $CD34^+$	Phase I: THPO, SCF, FLT3L, human stromal cells; Phase II: THPO, SCF, FLT3L, IL-11, human stromal cells; Phase III: THPO, SCF, FLT3L, IL-11	>0.5% $CD41^+$ megakaryocytes, 4.2×10^5 platelets/starting $CD34^+$ cell	[137]
UCB $CD34^+$	Phase I: THPO, SCF, IL-3; Phase II: THPO, IL-11	>85% $CD41^+CD42b^+$ megakaryocytes, 1.9×10^4 platelets/starting $CD34^+$ cell	[139]
UCB $CD34^+$	Phase I: THPO, SCF, FLT3L, IL-3, SR1; Phase II: THPO, SCF, IL-3, IL-6, IL-11, GM-CSF	>70% $CD41^+CD42b^+$ megakaryocytes	[138]
UCB $CD34^+$	Phase I: THPO, SCF, FLT3L, IL-6, SR1, C433, VPA; Phase II: THPO, SCF, IL-3, IL-6, IL-11, GM-CSF	>70% $CD41^+CD42b^+$ megakaryocytes	[140]

Megakaryopoiesis is controlled by TFs which turn on the expression of megakaryocyte lineage-specific genes and suppress the transcriptional programmes of other lineages [141–143]. TFs found to be involved in this process include RUNX1, FLI1, GABPA, GATA2, LMO2, MYB, and NFE2 [144,145]. There is evidence that megakaryocyte and erythroid progenitors share a common bipotential precursor. In agreement with this, they have several TFs in common, including GATA1, FOG1, TAL1, and GFI1B [146,147]. Megakaryocyte development is coordinated by the temporal expression of these TFs and mediated at multiple levels by different cytokines, including THPO and interleukins.

THPO: The most critical megakaryocyte-stimulating cytokine is THPO. THPO regulates megakaryocyte differentiation from the earliest stages of megakaryopoiesis, and all progenitors primed to become megakaryocytes, including HSCs, express the THPO

receptor MPL [148]. THPO is constitutively produced by the liver and sequestered by circulating platelets upon entry into the blood stream. When platelet count drops, for example, during haemorrhage, circulating levels of THPO increase and it enters the BM to stimulate megakaryopoiesis [149–151]. Loss of THPO signalling causes a drastic reduction in megakaryocytes and platelets [52]. However, patients with loss-of-function MPL mutations are still able to produce reduced numbers of platelets, suggesting that there is also a THPO-independent megakaryopoiesis pathway [152].

Interleukins (IL): The IL family comprises a disparate group of cytokines that are produced predominantly by leukocytes, and act as immunomodulators to elicit a variety of responses in cells and tissues throughout the body. They play a role in an array of processes, including cell proliferation, differentiation, migration and adhesion [153]. Interleukins that influence megakaryopoiesis include IL-1β and IL-6, which were found to upregulate THPO production resulting in increased platelet numbers in vivo [154,155], and IL-3, which was found to increase megakaryocyte proliferation in vitro without affecting maturation [156,157]. IL-1α has a THPO-independent function acting on mature megakaryocytes to promote shedding of proplatelets [158].

4. In Vitro Differentiation to Erythrocytes

Red blood cells (RBC), or erythrocytes, are the most abundant blood cell type, with ~2 × 10^6 new erythrocytes produced per second in adults [159,160]. Reduced levels of healthy RBCs can lead to anaemia, defined by the inability of blood to adequately oxygenate the body's tissues. Traditionally, the reduction of haemoglobin levels below 9–10 g/dL necessitates RBC transfusion [161,162]. RBC transfusion is conventionally employed in the treatment of haemorrhage, thalassaemia, chronic aplastic anaemia and chemotherapy/radiotherapy-induced anaemia, and is also used as supportive treatment for a range of genetic, autoimmune and neoplastic diseases. The demand for RBCs has continued to increase due the increasing burden of chronic disease and increasing severity of illness of intensive care patients brought about by an ageing population. The development of new in vitro methods for production could help to improve RBC supplies.

In vitro production of mature erythrocytes from HSCs utilises combinations of cytokines and/or stromal cells. It is commonly divided into several steps, which include erythroid lineage specification, erythroid progenitor expansion and erythroid maturation (Table 3). The purity of the cultures is commonly measured by the expression of the erythroid markers glycophorin A (CD235a), transferrin receptor (CD71) and/or Rhesus antigen (RhD), in combination with morphology analysis by May-Grünwald-Giemsa staining. One of the first protocols for erythroid differentiation of HSPCs utilised a 3-step process with specific cytokine combinations in serum-free culture medium to produce erythroid precursors that were capable of terminal maturation upon transplantation into immunodeficient mice [163]. In vitro terminal maturation and enucleation of erythroblasts was first achieved through the combined use of cytokines and co-culture on stromal cells [164]. Shortly after, stroma-free methods of producing erythroid cultures with incomplete but high percentages of mature/enucleated red blood cells were also developed [165]. As a proof-of-principal trial, a small number of RBCs generated from PB $CD34^+$ HSPCs using stroma-free conditions were transfused into a health volunteer to monitor the lifespan of cultured RBC in humans (NCT0929266) [166]. However, the generation of large-scale, fully mature and clinically applicable RBCs has remained an obstacle to researchers for a long time, with the first clinical trial to transplant donor HSC-derived RBC launched only recently (ISRCTN42886452). The long-standing issues with incomplete enucleation of cultured erythrocytes that has delayed clinical applications has not prevented the use of cultured erythroid cells as a model to study erythropoiesis and related disorders, as the protocols available yield a highly proliferative and pure culture. Recently, large-scale (roller-bottle cell culture system) generation of functional enucleated RBCs from UCB-derived HSPCs has been demonstrated to be a safe source of RBCs in xenotransfusion studies [167].

Table 3. Summary of protocols for in vitro erythroid differentiation.

Cell Source	Method	Cells Generated	Reference
UCB $CD34^+$	Phase I: SCF, THPO, FLT3L, hydrocortisone; Phase II: SCF, EPO, IGF-I, hydrocortisone; Phase III: EPO, IGF1, hydrocortisone	>80% $CD71^+CD235a^+$ erythrocytes	[163]
UCB and PB $CD34^+$	Phase I: SCF, IL3, EPO, hydrocortisone; Phase II: EPO in stromal cell co-culture; Phase III: stromal cell co-culture	>80% $CD71^+$ erythrocytes	[164]
UCB $CD34^+$	Phase I: SCF, EPO, IL-3 or SCF, EPO, IL-3, VEGF, IGF-II; Phase II: SCF, EPO; Phase III: SCF, EPO(reduced); Phase IV: plasmanate and mifepristone	>90% $CD235a^+RhD^+$ erythrocytes	[165]
PB $CD34^+$	Phase I: SCF, IL3, EPO, hydrocortisone; Phase II: SCF, EPO; Phase III: EPO	>85% $CD71^+CD235a^+$ erythrocytes	[166]
UCB $CD34^+$	Phase I: SCF, THPO, FLT3L; Phase II: SCF, FLT3L, EPO, IL3, GM-CSF; Phase III: SCF, FLT3L, EPO, IL3; Phase IV: SCF, EPO	>90% $CD235a^+$ erythrocytes	[167]

The complex transcriptional programmes that define erythropoiesis are coordinated by a set of potent TFs [168,169], the expression of which is capable of transdifferentiating non-erythroid cells into erythroid cells [170]. GATA1 is the predominant key erythroid TF critical for erythroid lineage commitment, and differentiation and loss of GATA1 activity leads to impaired erythropoiesis [171–177]. Another key erythroid TF, KLF1, acts first to suppress the megakaryocyte programme and promote erythroid fate in the early stages of differentiation [178,179], and later triggers cell-cycle exit and chromatin condensation during terminal erythroid maturation, just before enucleation [180–184]. There are many more erythroid-associated TFs which form complex networks of coactivators or corepressors to modulate target gene expression. These include another major player, TAL1, which, along with LMO2 and LDB1, form a co-activator complex that binds GATA1 to activate the transcription of erythroid-associated genes [168,185]. Erythroid TFs are a downstream target of conserved signalling pathways which are activated by a number of extrinsic factors that control the differentiation, proliferation and survival of erythroid cells. The major erythropoietic cytokine is erythropoietin (EPO). However, other cytokines have been shown to promote erythroid proliferation and survival, including SCF [186,187] and IL-3 [188,189] (discussed above).

Erythropoietin (EPO): EPO is the most critical erythroid-stimulating cytokine. Human EPO was cloned in 1984 [190,191], and acts to promote the survival and proliferation of erythroid cells, starting with the earliest immature erythroid progenitors and persisting through to later stages of maturation. However, the EPO receptor (EPOR) is weakly expressed on erythroid cells, and its expression quickly decreases with terminal maturation [192–195]. EPO binding leads to EPOR dimerisation and activation of JAK/STAT to induce the erythroid transcriptional programme. Other signalling pathways stimulated by EPO include the MAPK and PI3K pathways, which act to promote survival and/or proliferation [196–199]. There is evidence to suggest that EPO may prime erythroid commitment in HSPCs, and that EPOR may be more broadly expressed in HPCs, indicating that erythroid lineage commitment may occur early during haematopoiesis [195,200–202]. EPO is produced in the liver and adult kidney, and acts to regulate the level of oxygen in the blood by modulating the number of circulating erythrocytes [203–208].

5. In Vitro Differentiation into Myeloid Cells

The myeloid compartment includes granulocytes (neutrophils, eosinophils and basophils), monocytes (macrophages and dendritic cells) and mast cells [209]. As the most numerous and extensively studied cells, we focus here on neutrophils and monocytes. Myeloid lineage specification is governed primarily by the C/EBP family and PU.1 TFs [210–212]. In mice, a high PU.1 concentration first regulates the decision between lymphoid and myeloid fates [213,214]. C/EBPα is then expressed in immature myeloid cells [215] and is critical for the CMP to GMP transition [216]. In myeloblasts, PU.1 and C/EBPα oppose each other's expression, and a high C/EBPα/PU.1 ratio promotes granulopoiesis over monopoiesis [215,217,218]. C/EBPα then instructs neutrophil differentiation by activating the TF GFI1, whereas PU.1 activates IRF8, KLF4 and EGR2 to mediate monocytic differentiation [212,219–222]. Finally, C/EBPε is mainly expressed in late granulopoiesis and mediates neutrophil maturation [212,215].

5.1. Neutrophils

Neutrophils are the most abundant leukocyte in the blood, but have a lifespan of only 12–14 h, meaning that ~10^{11} neutrophils must be produced daily by the BM. Neutrophils play a key role in the innate control of infection by killing bacterial and fungal cells using phagocytosis and by releasing cytotoxic granules (degranulation) or nuclear material (neutrophil extracellular traps) [223,224]. Neutropenia is common following viral infection as a result of leukaemia and following chemotherapy or HSCT, and is associated with significant mortality due to the inability to control opportunistic infections [225,226]. Infusion of G-CSF or corticosteroid mobilised mature granulocytes has proven ineffective at reducing neutropenia, potentially due to insufficient cell dose or short intravascular residence time [227,228]. Infusions of non-HLA matched myeloid progenitors are undergoing clinical trials (NCT02282215, NCT01297543, NCT00891137, NCT01690520, NCT01175785, NCT01031368, NCT03399773, NCT04083170) as a treatment to reduce pathologies associated with neutropenia, such as the number of febrile days or microbiologically defined infections [227,229]. Thus, numerous protocols have been developed to expand neutrophils ex vivo, often with the aim of eventual clinical translation. Differentiation towards the granulocyte lineage is typically achieved with a combination of SCF, FLT3L, IL-3, GM-CSF and G-CSF [230–235] (see Table 4).

Early differentiation protocols often produced cells that were less than 50% immunophenotypic neutrophils [63,236], which are typically identified using a combination of Sialyl-Lewis X (CD15), carcinoembryonic antigen-related cell adhesion molecule 8 (CD66b) and/or FcγRIII (CD16). Subsequent protocols have aimed to improve purity. The use of protocols organised into stages first allows rapid expansion of myeloid progenitors with combinations of SCF, IL-3 or FLT3L, followed by a minimal set of lineage-defining cytokines, such as GM-CSF or G-CSF, which restricts differentiation towards the neutrophil lineage [231–233]. THPO or THPO mimetics can be included [63,237], but others report that THPO does not enhance growth of neutrophil progenitors [230,232] or can impair neutrophil differentiation [238]. Choi et al.[239] also noted that SCF and/or FLT3L minimally promote proliferation of myeloid precursors, but significantly increase the proportion of $CD235a^+$ erythroid cells. Finally, switching from use of serum to serum albumin to supplement medium can also reduce multi-lineage differentiation [232]. Modern protocols are now capable of producing neutrophils demonstrating key functional characteristics such as chemotaxis, phagocytosis, oxidative burst, neutrophil extracellular trap formation and bactericidal activity at levels comparable PB neutrophils [232,233].

IL-3 and GM-CSF: IL-3 and GM-CSF promote the survival, differentiation and proliferation of a range of myeloid progenitor cells [236,240,241]. Indeed, the expression of IL-3 receptor (IL-3R, or CD123) is itself a marker for the common myeloid progenitor and granulocytic/monocytic progenitor cell identity [242]. Treatment of $CD34^+$ HSPCs with IL-3 and/or GM-CSF induced proliferation and differentiation into cell types expressing early and late myeloid markers [236]. The IL-3R and GM-CSF receptor (GM-CSFR, or

CSF2) are members of the type I cytokine receptor family, and share a common beta subunit [243–245]. Thus, engagement of either receptor triggers common downstream signal transduction pathways, including JAK2/STAT5, JNK-MAPK, PI3K and NFkB signalling. Signalling transduction downstream of IL-3R and GM-CSFR are extensively reviewed elsewhere [243,244].

G-CSF: G-CSF is a critical cytokine for neutrophil survival, proliferation and differentiation [228,234,246,247]. However, G-CSF only promotes high levels of granulocyte proliferation in synergy with SCF, FLT3L or IL-3 [232,236]. C/EBPε is induced only after addition of G-CSF to culture medium [248], and thus mature $CD16^+$ neutrophils do not appear in liquid culture without the addition of G-CSF [63,248]. G-CSF influences the state of a myeloid progenitor by binding G-CSF receptor (G-CSFR, also known as CSF3R) and inducing its dimerization. G-CSFR lacks intrinsic kinase activity, but as a dimer it complexes with and activates protein tyrosine kinases, including JAK1/2, TYK2, SRC family kinases and SYK [249–251], which further activate MAPK/ERK and PI3K signalling [249,252,253]. JAK/STAT activation proceeds mainly via JAK1/2 and STAT1/3 [249,254], although STAT5 is also necessary for G-CSF induced differentiation in murine cells [255]. G-CSF mediated ERK1/2 activation is transient, and, paradoxically, ERK1/2 inhibition can direct differentiation towards the neutrophil rather than monocyte lineage [256,257]. Phosphorylation of C/EBPε and β by MAPK regulates expression of secondary granule genes and inhibits apoptosis [258], although the role of all G-CSFR dependent signalling is not yet fully understood. Signal transduction downstream of G-CSFR is reviewed elsewhere [251].

Table 4. Summary of protocols for in vitro neutrophil differentiation.

Cell Source	Method	Cells Generated	Reference
UCB $CD34^+$	THPO, FLT3L, G-CSF	6–23% $CD16^+$ ($CD32^{hi}CD64^{hi}$)	[63]
UCB or BM $CD34^+$	IL-3, GM-CSF, G-CSF, M-CSF	100× expansion, 10–70% $CD33^+$, <20% $CD14/CD15^+$	[236]
PB $CD34^+$ PBMCs	SCF, IL-3, GM-CSF, G-CSF	130–220× expansion, by morphology: 65% granulocytic, 11% band/segmented neutrophils, 5% monocyte/macrophages	[231]
UCB $CD34^+$ HSPCs	SCF, IL-3, FLT3L, G-CSF	60–70% $CD15^+$, 75% MPO^+	[230]
G-CSF mobilised PB $CD34^+$	SCF, FLT3L, G-CSF	30× expansion, 80% mature neutrophils (by morphology), $CD16b^{lo}$,	[238]
BM $CD34^+$	SCF, THPO, IL-3, G-CSF	76% band/segmented neutrophils, $CD15^+CD66^+$	[259]
UCB $CD34^+$	SCF, G-CSF, THPO mimetic	5800× expansion, 61% metamyelocytes/band/segmented neutrophils, 73% $CD15^+$	[237]
UCB $CD34^+$	Phase I: SCF, IL-3, FLT3L, GM-CSF; phase II: G-CSF	8900–49,000× expansion, 59% $CD66b^+$	[232]
UCB $CD34^+$	SCF, FLT3L, IL3, THPO, EPO, with MS-5 stromal cells	Pan-myeloid differentiation, 23% $CD34^+$, 12% $CD14^+$ monocytes, 5% $CD66b^+$ granulocytes, 8% $CD41^+$ megakaryocytes, 11% $CD235a^+$ erythrocytes	[240]
UCB or PB $CD34^+$	Staged combinations of SCF, IL-3, FLT3L, GM-CSF, G-CSF	50–70× expansion, 70–92% $CD15^+$, 43–57% $CD66b^+$	[233]

5.2. Monocytes

Monocytes are a heterogenous cell population that play a key role in innate immunity, wound healing and chronic inflammation [260]. The most common source of monocytes (or subsequently in vitro differentiated macrophages) remains peripheral blood mononuclear cells (PBMCs), but this has limited their study or clinical use, as only a small proportion

of PB monocytes are capable of proliferation in vitro [261]. Infusions of autologous monocytes/macrophages have been tested as a tumour treatment since the late 80s, but regardless of dose, schedule or cell source, only a partial response was seen in a few patients [227,262]. Testing of monocyte infusion continues for tumours such as ovarian carcinoma, where immune checkpoint inhibition shows limited efficacy (NCT02948426) [263,264]. Monocyte or macrophage infusions are also being developed as a treatment for inflammatory diseases such as liver cirrhosis (EudraCT number 2015-000963-15) [265,266] and ischaemic stroke (NCT024335090) [267].

In recent years, several methods for the in vitro differentiation of monocytes have been developed and may facilitate a greater understanding of monocyte biology. These typically use a combination of Siglec-3 (CD33), FcγRI (CD64), the lipopeptide receptor CD14 and/or CD16 as monocyte markers to assess culture purity. The cytokines IL-6 and M-CSF direct HSPCs towards the monocyte lineage, and IL-6 specifically favours macrophage rather than dendritic cell differentiation [268,269]. Early protocols using M-CSF and IL-6 induced differentiation of PB-derived $CD34^+$ HSPCs into the monocyte lineage without expansion [270], but M-CSF or IL-6 alone (or in combination with other cytokines) do not promote proliferation [271]. Subsequent inclusion of early-acting cytokines such as SCF, IL-3, or FLT3L then supported monocyte expansion alongside differentiation (see Table 5). The addition of vitamin D3 can also bias the specific monocyte subsets produced [272], and the use of IL-2 can generate novel minor subsets ($CD56^+$ $CD33^+$) of monocytes [273]. Macrophages produced from the most recent protocols have gene expression profiles similar to PB monocytes and demonstrate key phenotypes including phagocytosis, adhesion and osteoclast generation [271,274].

Monocytes can be further differentiated into dendritic cells or polarised towards different sets of macrophages, but this will not be discussed in depth here. Briefly, M1 pro-inflammatory macrophages can be generated with lipopolysaccharide (LPS), IFN-γ or GM-CSF [234,275,276], whereas various M2 subtypes can be generated using combinations of IL-4, IL-10, IL-13, glucocorticoids, IL-1β or LPS [234,275]. The dendritic cell lineage is typically established using GM-CSF and IL-4 [241,244,277,278].

M-CSF: M-CSF is a potent factor driving the survival and differentiation of monocytes and macrophages [279–281]. Enforced expression of the M-CSF receptor, M-CSFR (also known as CSF-1R, or CD115), in UCB-derived $CD34^+$ or even B cells promotes monocyte differentiation in vitro and in vivo [282,283]. M-CSFR is a class III receptor tyrosine kinase [251], which, upon activation, complexes with and phosphorylates GRB2/SOS, the p85 subunit of PI3K, SFK and CBL [284–287]. Activation of the PI3K/AKT pathway promotes survival, motility and proliferation in macrophages [286,288–290]. In mouse models, M-CSF stimulates more potent and sustained activation of Erk1/2, which is necessary for monocyte development and proliferation [257,289–292]. M-CSF dependent Src kinase activity instructs the monocyte fate by inducing expression of PU.1 [280], although the specific roles of the other signalling pathways are not yet completely described. Downstream signalling is reviewed elsewhere [287].

Table 5. Summary of protocols for in vitro monocyte differentiation.

Cell Source	Method	Cells Generated	Reference
G-CSF mobilised PB $CD34^+$	M-CSF, mast cell growth factor (MGF), IL-6	No expansion, 55% $CD33^+CD14^+$, 62% $CD33^+$HLA-DR^+	[270]
G-CSF mobilised PB $CD34^+$	SCF and IL-2	Majority CD33+, 2.5% $CD33^+CD56^{dim}$, NK-like monocytes	[273]
UCB $CD34^+$	SCF, IL-3, FLT3L and M-CSF	300× expansion, 45% $CD14^+$, 22% $CD16^+$	[272]
G-CSF mobilised PB $CD34^+$	SCF, FLT3L, IL-3, IL-6, GM-CSF, M-CSF with 0.5% buminate	360× expansion, 90% $CD11b^+$, 65% $CD64^+$, 45% $CD14^+$	[278]
G-CSF mobilised PB	SCF, FLT3L, IL-3, IL-6 M-CSF with 1% Buminate	90% lysozyme positive, $CD14^+CD64^+CD16^+$HLA-DR^+	[271]
UCB $CD34^+$	SCF, IL-6, FLT3L or SCF, THPO and FLT3L and viral transduction of M-CSF	350× expansion, 70–80% $CD33^+CD14^+$	[283]

6. In Vitro Differentiation into Lymphoid Cells

In the classical model for lymphoid differentiation, HSCs differentiate into LMPPs and then into CLPs, which then commit to B, T or natural killer (NK) cell lineages. Here, we will focus on the differentiation into B and T cells, given the larger numbers of studies into these lineages.

6.1. B Cells

Mature B cells are characterized by surface expression of mature immunoglobulin (Ig), produced from successfully recombined heavy and light chain loci. Post-natal B cell lymphopoiesis occurs in the BM in vivo. In humans, several B cell precursors have been identified. CLPs ($CD34^+CD38^+CD45RA^+CD10^+$) differentiate into pro-B cells ($CD34^+CD10^+CD19^+IgM^-$), then pre-B cells ($CD34^-CD10^+CD19^+IgM^+$), then immature B cells ($CD34^-CD19^+IgM^+$) and finally mature B cells [293,294]. Pro-B cells are the earliest committed B cell population, and the first to undergo Ig heavy chain rearrangement. It has become well-established that the entry into B cell fate depends on the activation of four key TFs (E2A, EBF1, FOXO1 and PAX5 [295]), which act on each other in a stepwise fashion. In CLPs, the E2A gene product E47 is essential in normal lymphocyte development as well as in B lymphocyte V(D)J recombination [296]. E2A, in turn, acts on EBF1 and FOXO1; EBF1 acts on FOXO1; and E2A, EBF1 and FOXO1 all act on PAX5 [295]. PAX5, in turn, has been found to be a master regulator TF which simultaneously represses inappropriate B cell lineage genes and activates B cell lineage-specific genes [297]. Several culture systems have been developed to support in vitro B cell development (see Table 6), but generally require integrin signalling and IL-7.

Integrins: Leukocyte integrins have long been known to play essential roles in localization, activation and differentiation of human lymphocytes, and the first paper to identify an essential component of B cell differentiation focused on BM stromal contact. In 1990, it was shown that murine pre-B cells adhere to fibronectin, but lose this ability as they mature [298]. Notably, however, it was found soon after that human B cell precursors require VLA-4/VCAM-1 interaction (not the VLA-4/fibronectin interaction) for long-term development. While the exact mechanism remains unclear, subsequent studies have shown that cross-linking of VLA-4 with VCAM-1 leads to induction of tyrosine kinase pathways in B cells [299,300]. Notably, a more recent protocol for feeder-free B cell differentiation used immobilized ICAM1-Fc-coated plates. However, this publication attributed the use of ICAM-1 (which binds LFA-1 expressed on $CD34^+$ HSPCs and B cells) to promote development of lymphoid progenitors, and found that differentiation into B cells was independent

of ICAM-1 [301], demonstrating that differentiation of $CD34^+$ HSPCs into B cells is possible even without contact elements.

IL-7: Following the discovery that B cells could be cultured with stromal cells, the first stromal cell growth factor isolated was IL-7 (found by screening a cDNA library prepared from a stromal cell line [302]), and IL-7 quickly made its way into a staple stromal culture supplement [303]. Thereafter, it was found that IL-7 promotes B cell clonal proliferation (but not differentiation) [304], and it was found that IL-7 specifically promoted proliferation of pro-B cells but not pre-B cells [305]. Closer investigation into the IL-7 receptor has revealed that it possesses a number of functional intracellular domains, including SRC family-related regions, JAK/STAT related regions and PI3K/AKT activation regions [306], indicating a wide range of downstream functions related to proliferation. CLPs from Il-7-knockout mice have been shown to have impaired B cell generation ability, likely due to the observed lower expression of key B cell TFs EBF and PAX5 [307]. However, ectopic overexpression of EBF restores B cell development [307,308], indicating EBF to be a primary downstream target of IL-7. Further investigations have revealed induction of EBF1 through the JAK/STAT5 signalling pathway [308]. Notably, FLT3L has also been found to act in synergy with IL-7, albeit it through independent signalling pathways [309].

Other factors: In addition to SCF, FLT3L and IL6 (all introduced above), additional cytokines including IL-4 [310] have been found to promote B cell lymphopoiesis. However, it is likely that they exert their influence primarily in latter phases of B cell development, as is the case for IL-2, IL-5 and IL-10 [311–313]. Additionally, it has been found that Activin A and TGF-β1 are negative regulators for early B cell lymphopoiesis, and antibodies and inhibitors targeting these factors can increase B cell lymphopoiesis [314]. Notably, as many in vitro cultures are successful in generating B cell precursors even in the absence of these cytokines and factors, it is likely that these cytokines are not necessary for B cell lineage commitment.

Table 6. Summary of protocols for in vitro B cell differentiation.

Cell Source	Method	Cytokines	Cell Generated	References
Nucleated BM cells	BM fibroblast feeder layer	None	$CD10^+/CD20^+$ B-lineage cells	[315]
UCB $CD34^+$	Murine fetal stromal feeder cell layer (MS-5) with cytokines	SCF, G-CSF	$CD19^+/IgM^+$	[316]
BM $CD34^+Lineage^-CD38^-$	Murine fetal stromal feeder cell layer (AFT024) with cytokines	IL-2, SCF, FLT3L, IL-7, IL-3	$CD10^+/CD19^+$ B-lineage cells	[317]
Fetal BM $CD34^+Lineage^-$	Human fetal BM stromal cells	None	IgM^+ immature B cells	[318]
UCB $CD34^+$	Human BM stromal cell layer with cytokines (and additional antibodies)	SCF, FLT3L; (anti-Activin A and anti-TGFβ antibodies)	IgM^+ immature B cells	[314]
UCB $CD34^+$	Murine stromal cell layer (S17)	Il-10, IL-4, FLT3L, IL-2	IgM/IgG-secreting B cells	[319,320]
Fetal liver $CD34^+$	Murine stromal cell (OP9) with cytokines	TSLP	Mature $CD34^-CD38^+CD19^+IgM^+IgD^+$ B cells	[321]
UCB or BM $CD34^+$	ICAM-1 coated plate with cytokines	IL-6, SCF, FLT3L, IL-7	IgM^+ $CD19^+$ immature B cells	[301]
UCB $CD34^+$	Cytokines only	IL-6, FLT3L, SCF, IL-7	$CD10^+CD79\alpha^+CD19^+$ pro-B cells	[322]

6.2. T Cells

The development of T cells in humans occurs primarily in the thymus and requires carefully mediated migration of T cell progenitors into thymic regions, which provide a specialised microenvironment for T cell differentiation. The earliest T cell precursors

in the thymus are known as early thymic progenitors (ETPs) or double negative 1 (DN1) cells, defined as $CD34^+CD7^-CD5^-CD1a^-$ cells. As ETPs differentiate, they generate DN2 cells, which are committed to the T cell lineage. Notably, RAG-mediated rearrangement of the T cell receptor (TCR) is detectable in the DN2 phase, but presents predominantly in the DN3 ($CD7^+CD5^+CD45RA^+$) phase, during which only cells with a productively rearranged TCR-beta and successfully formed pre-TCR complex are permitted to continue differentiation along the alpha/beta lineage. Subsequently, these cells become DN4 (or pre-DP cells), then immature single positive (ISP) cells (named for brief CD4 expression), then $CD4^+CD8^+$ double positive (DP) cells, which undergo TCR-alpha rearrangement and result in a completely assembled TCR. DP cells then undergo positive selection for MHC binding, then negative selection for autoreactivity, before finally becoming mature $CD4^+$ or $CD8^+$ single positive (SP) T cells.

One of the most critical signals is activation of the TF TCF1 (encoded by *TCF7*), which then results in subsequent expression of many T cell-specific TFs such as GATA3 and BCL11B [323]. Expression of GATA3 represses FLT3 expression, causing loss of B cell potential; similarly, activation of BCL11B (regulated in part by GATA3) prevents NK cell differentiation, and myeloid/DC potential is lost by PU.1 silencing. Activation of these three TFs (TCF1, GATA3, and BCL11B) allows progression from ETP stage through to DN2, upon which a host of other TFs (such as RUNX1/CBFB, GFI1, E2A, MYB and IKAROS) solidify the commitment to the T cell lineage [324].

T cells have important applications in cancer treatment, particularly as tumour-infiltrating lymphocytes (TILs) and chimeric antigen receptor (CAR) T cells. CAR T cell therapies require the production of large number of T cells; however, current methods of T cell production suffer from several problems. These include (1) peripheral blood T cells sourced for treatment often exhibit compromised (e.g., anergic or exhausted) function [325], and (2) the need for T cells to be sourced autologously prevents production of T cell therapies at scale. Protocols allowing for the in vitro differentiation of T cell precursors from HSCs could solve these problems [326]. Several culture systems have been developed to support in vitro T cell development (see Table 7), a process that is highly dependent on Notch signalling.

Notch signalling: One of the earliest discovered requirements for T cell differentiation was Notch signalling. Upon migration to the thymus, the thymic microenvironment provides the critical Notch ligand DLL4, which triggers proteolytic release of intracellular NOTCH1. Recent genome-wide studies have indicated that Notch signalling is related to many targets, but prominent targets include MYC, DTX1 and members of the HES and HRT family, as well as crosstalk with other signalling pathways including NFkB and hypoxia [327–331]. In particular, Notch signalling induced TCF1 expression, which, in turn, drives expression of key T cell TFs such as GATA3 and BCL11B [323]. As discussed below, various approaches have been used to stimulate Notch signalling within in vitro T cell differentiation culture systems.

Stromal co-culture-based T cell differentiation: The earliest T cell cultures used OP9 murine BM stromal cells retrovirally transduced with Notch ligand Delta-like-1 (DLL1) to create the OP9-DLL1 cell line. Murine fetal liver haematopoietic progenitor cells cultured on OP9-DLL1 were shown to support robust differentiation to DP T cells and some generation of mature SP T cells [332]. The culture system has since been successfully expanded to human UCB-derived HSPCs [333], and pro-T cells derived from these cultures can engraft in immune-deficient mice [334]. Notably, however, the T cells differentiated on OP9-DLL1 cells demonstrate high bias towards the $CD8^+$ T cell lineage [335]. A second DLL1 expressing stromal cell type, the TSt-4 stromal line, is also used to support T cell differentiation from UCB-derived HSPCs [336]. Notably, while the OP9-DLL1 system remains widely used, subsequent studies have found DLL4 (and not DLL1) to be the critical Notch ligand, as DLL4 allows more efficient T cell differentiation [337], and DLL1 deletion (but not DLL4 deletion) [338] from thymic epithelial cells has no effect on T cell lymphopoiesis.

Artificial Thymic Organoid (ATO)-based T cell differentiation: ATO cultures are characterized by a 3D aggregate of mixed HSPCs and thymic cells, cultured on top of a porous membrane and placed into liquid media [339–342]. As with stromal cell monolayers [335], ATO differentiation has been demonstrated to generate mature naïve SP T cells [342]. However, the efficiency of ATO differentiation into mature T cells has been found to outperform stromal cell monolayers [342]. While the reason behind the improved efficiency remains unknown, it is possible that the 3-dimensional nature of the ATO increases the heterogeneity and likelihood of thymic-like niches, which may facilitate movement of T cells towards regions within the ATO which promote T cell maturation. Furthermore, the increased density between T cells and stromal cells in ATOs may also promote crosstalk, which enhances T cell maturation.

Stromal-free T cell differentiation: Due to inherent variability introduced into cell culture from the use of feeder cells, several attempts have been made to differentiate HSCs into T cells using feeder-free culture. The essential component of these feeder-free cultures has been the use of Notch ligands fused to the antibody Fc region, and subsequent immobilization onto a plate [343,344]. Immobilized DLL4 cultures have been validated for UCB and mPB HSPC differentiation [72,345,346]. However, these studies also highlighted differences between immobilized DLL4 cultures and in vivo thymic development, namely that development seemed blocked at the pre-T cell phase. Nevertheless, transplanted DLL4-cultured T cells still successfully engraft in vivo [345]. Additional work on optimizing feeder-free cultures has identified a range of molecules which appear to boost T cell development, such as WNT3A [347], ascorbic acid [348] and VCAM-1 [349]. More recently, use of DLL4-microbeads instead of immobilized DLL4 has allowed conversion from a plate-based system to a bioreactor system, allowing production of clinically relevant numbers of T cells [350]. Notably, while the system demonstrated limited progression to the SP T cell phase, successful thymic engraftment into immunodeficient mice was observed, which paves the way for clinical translation.

Other factors: Other factors found to have an influence on T cell lymphopoiesis include IL-7, SCF, IL-3, IL-15, CXCL12 and TNFa. While IL-3 alone appears to stimulate myeloid-biased differentiation, when paired with TNFa there appears to be a strong proliferative effect towards lymphoid-biased differentiation [351]. CXCL12 (which binds CXCR4) has been found to be expressed on TECs [352], and promotes Notch-dependent differentiation [353]. Notably, a number of additional cytokines (such as IL-2 and IL-15) have also been implicated in proliferation of mature T cells [354,355].

Table 7. Summary of protocols for in vitro T cell differentiation.

Cell Source	Method	Cytokines	Cell Generated	Reference
UCB $CD34^+CD38^-$	OP9-DLL1 stromal feeder cell with cytokines	FLT3L, IL-7	$CD4^+CD8^+$ DP T cells	[333]
UCB $CD34^+CD38^-CD3^-CD19^-$	Culture on MS5 then transfer to DLL4-coated plate	IL-2, IL-15, SCF	$CD7^+CD3^+$	[356]
UCB $CD34^+CD38^-$	Murine stromal feeder layer (Tst-4/hDLL1)	None	$CD5^+CD7^+$ immature T cells	[336]
UCB $CD34^+$	DLL4-coated plate with cytokines	SCF, THPO, FLT3L, IL-7	$CD5^+CD7^+CD1a^+$ immature T cells	[345]
UCB $CD34^+$	DLL4- and VCAM-1 coated plate with cytokines	SCF, THPO, FLT3L, IL-7	$CD7^+$ pro-T cells	[349]
UCB $CD34^+$	DLL4- and VCAM-1 coated plate with cytokines	SCF, THPO, FLT3L, IL-7, IL-3, TNFa	$CD4^+CD8^+$ DP T cells	[351]
UCB and BM $CD34^+$	ATO system with cytokines	FLT3L, IL-7	$CD4^+$ and $CD8^+$ mature SP T cells	[342]
UCB $CD34^+$	DLL4-coated microbeads with cytokines	FLT3L, SCF, IL-7	$CD7^+CD5^+$ immature T cells	[350]

7. Summary

A large research effort over the last five decades has led to a range of in vitro human HSPC maintenance, expansion and differentiation protocols. These methods provide useful models to study human haematopoiesis and generate cell products for cell therapies. However, current technical challenges in stably expanding pure populations of bone fide human HSCs in vitro limit the further development of these technologies to comprehensively study human haematopoiesis in a dish. By contrast, the establishment of stable in vitro culture conditions for the expansion and differentiation of embryonic stem cells has enabled major insights into human development. Further improvements in expansion and differentiation culture conditions for human HSCs should extend the utility of these systems.

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References

1. Boulais, P.E.; Frenette, P.S. Making Sense of Hematopoietic Stem Cell Niches. *Blood* **2015**, *125*, 2621–2629. [CrossRef]
2. Pinho, S.; Frenette, P.S. Haematopoietic Stem Cell Activity and Interactions with the Niche. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 303–320. [CrossRef] [PubMed]
3. Morrison, S.J.; Scadden, D.T. The Bone Marrow Niche for Haematopoietic Stem Cells. *Nature* **2014**, *505*, 327–334. [CrossRef] [PubMed]
4. Notta, F.; Doulatov, S.; Laurenti, E.; Poeppl, A.; Jurisica, I.; Dick, J.E. Isolation of Single Human Hematopoietic Stem Cells Capable of Long-Term Multilineage Engraftment. *Science* **2011**, *333*, 218–221. [CrossRef]
5. Laurenti, E.; Göttgens, B. From Haematopoietic Stem Cells to Complex Differentiation Landscapes. *Nature* **2018**, *553*, 418–426. [CrossRef]
6. Boitano, A.E.; Wang, J.; Romeo, R.; Bouchez, L.C.; Parker, A.E.; Sutton, S.E.; Walker, J.R.; Flaveny, C.A.; Perdew, G.H.; Denison, M.S.; et al. Aryl Hydrocarbon Receptor Antagonists Promote the Expansion of Human Hematopoietic Stem Cells. *Science* **2010**, *329*, 1345–1348. [CrossRef]
7. Eaves, C.J. Hematopoietic Stem Cells: Concepts, Definitions, and the New Reality. *Blood* **2015**, *125*, 2605–2613. [CrossRef] [PubMed]
8. Lehnertz, B.; Chagraoui, J.; MacRae, T.; Tomellini, E.; Corneau, S.; Mayotte, N.; Boivin, I.; Durand, A.; Gracias, D.; Sauvageau, G. *HLF* Expression Defines the Human Hematopoietic Stem Cell State. *Blood* **2021**, *138*, 2642–2654. [CrossRef]
9. Christodoulou, C.; Spencer, J.A.; Yeh, S.-C.A.; Turcotte, R.; Kokkaliaris, K.D.; Panero, R.; Ramos, A.; Guo, G.; Seyedhassantehrani, N.; Esipova, T.V.; et al. Live-Animal Imaging of Native Haematopoietic Stem and Progenitor Cells. *Nature* **2020**, *578*, 278–283. [CrossRef]
10. Calvanese, V.; Nguyen, A.T.; Bolan, T.J.; Vavilina, A.; Su, T.; Lee, L.K.; Wang, Y.; Lay, F.D.; Magnusson, M.; Crooks, G.M.; et al. MLLT3 Governs Human Haematopoietic Stem-Cell Self-Renewal and Engraftment. *Nature* **2019**, *576*, 281–286. [CrossRef]
11. Xiang, P.; Wei, W.; Hofs, N.; Clemans-Gibbon, J.; Maetzig, T.; Lai, C.K.; Dhillon, I.; May, C.; Ruschmann, J.; Schneider, E.; et al. A Knock-in Mouse Strain Facilitates Dynamic Tracking and Enrichment of MEIS1. *Blood Adv.* **2017**, *1*, 2225–2235. [CrossRef] [PubMed]
12. Frelin, C.; Herrington, R.; Janmohamed, S.; Barbara, M.; Tran, G.; Paige, C.J.; Benveniste, P.; Zuñiga-Pflücker, J.-C.; Souabni, A.; Busslinger, M.; et al. GATA-3 Regulates the Self-Renewal of Long-Term Hematopoietic Stem Cells. *Nat. Immunol.* **2013**, *14*, 1037–1044. [CrossRef] [PubMed]
13. Lawrence, H.J.; Christensen, J.; Fong, S.; Hu, Y.-L.; Weissman, I.; Sauvageau, G.; Humphries, R.K.; Largman, C. Loss of Expression of the Hoxa-9 Homeobox Gene Impairs the Proliferation and Repopulating Ability of Hematopoietic Stem Cells. *Blood* **2005**, *106*, 3988–3994. [CrossRef]
14. Buske, C. Deregulated Expression of HOXB4 Enhances the Primitive Growth Activity of Human Hematopoietic Cells. *Blood* **2002**, *100*, 862–868. [CrossRef] [PubMed]

15. Sauvageau, G.; Thorsteinsdottir, U.; Eaves, C.J.; Lawrence, H.J.; Largman, C.; Lansdorp, P.M.; Humphries, R.K. Overexpression of HOXB4 in Hematopoietic Cells Causes the Selective Expansion of More Primitive Populations in Vitro and in Vivo. *Genes Dev.* **1995**, *9*, 1753–1765. [CrossRef]
16. Staal, F.J.T.; Chhatta, A.; Mikkers, H. Caught in a Wnt Storm: Complexities of Wnt Signaling in Hematopoiesis. *Exp. Hematol.* **2016**, *44*, 451–457. [CrossRef]
17. Nusse, R.; Clevers, H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* **2017**, *169*, 985–999. [CrossRef]
18. Pajcini, K.V.; Speck, N.A.; Pear, W.S. Notch Signaling in Mammalian Hematopoietic Stem Cells. *Leukemia* **2011**, *25*, 1525–1532. [CrossRef]
19. Siebel, C.; Lendahl, U. Notch Signaling in Development, Tissue Homeostasis, and Disease. *Physiol. Rev.* **2017**, *97*, 1235–1294. [CrossRef]
20. Blank, U.; Karlsson, G.; Karlsson, S. Signaling Pathways Governing Stem-Cell Fate. *Blood* **2008**, *111*, 492–503. [CrossRef]
21. Reya, T.; Duncan, A.W.; Ailles, L.; Domen, J.; Scherer, D.C.; Willert, K.; Hintz, L.; Nusse, R.; Weissman, I.L. A Role for Wnt Signalling in Self-Renewal of Haematopoietic Stem Cells. *Nature* **2003**, *423*, 409–414. [CrossRef] [PubMed]
22. Helgason, C.D.; Antonchuk, J.; Bodner, C.; Humphries, R.K. Homeostasis and Regeneration of the Hematopoietic Stem Cell Pool Are Altered in SHIP-Deficient Mice. *Blood* **2003**, *102*, 3541–3547. [CrossRef] [PubMed]
23. Bhatia, M.; Bonnet, D.; Kapp, U.; Wang, J.C.Y.; Murdoch, B.; Dick, J.E. Quantitative Analysis Reveals Expansion of Human Hematopoietic Repopulating Cells after Short-Term Ex Vivo Culture. *J. Exp. Med.* **1997**, *186*, 619–624. [CrossRef] [PubMed]
24. Conneally, E.; Cashman, J.; Petzer, A.; Eaves, C. Expansion *in Vitro* of Transplantable Human Cord Blood Stem Cells Demonstrated Using a Quantitative Assay of Their Lympho-Myeloid Repopulating Activity in Nonobese Diabetic– *Scid/Scid* Mice. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9836–9841. [CrossRef] [PubMed]
25. Walasek, M.A.; van Os, R.; de Haan, G. Hematopoietic Stem Cell Expansion: Challenges and Opportunities: HSC Expansion: Challenges and Opportunities. *Ann. N. Y. Acad. Sci.* **2012**, *1266*, 138–150. [CrossRef]
26. Himburg, H.A.; Termini, C.M.; Schlussel, L.; Kan, J.; Li, M.; Zhao, L.; Fang, T.; Sasine, J.P.; Chang, V.Y.; Chute, J.P. Distinct Bone Marrow Sources of Pleiotrophin Control Hematopoietic Stem Cell Maintenance and Regeneration. *Cell Stem Cell* **2018**, *23*, 370–381.e5. [CrossRef]
27. Himburg, H.A.; Yan, X.; Doan, P.L.; Quarmyne, M.; Micewicz, E.; McBride, W.; Chao, N.J.; Slamon, D.J.; Chute, J.P. Pleiotrophin Mediates Hematopoietic Regeneration via Activation of RAS. *J. Clin. Investig.* **2014**, *124*, 4753–4758. [CrossRef]
28. Himburg, H.A.; Harris, J.R.; Ito, T.; Daher, P.; Russell, J.L.; Quarmyne, M.; Doan, P.L.; Helms, K.; Nakamura, M.; Fixsen, E.; et al. Pleiotrophin Regulates the Retention and Self-Renewal of Hematopoietic Stem Cells in the Bone Marrow Vascular Niche. *Cell Rep.* **2012**, *2*, 964–975. [CrossRef]
29. Himburg, H.A.; Muramoto, G.G.; Daher, P.; Meadows, S.K.; Russell, J.L.; Doan, P.; Chi, J.-T.; Salter, A.B.; Lento, W.E.; Reya, T.; et al. Pleiotrophin Regulates the Expansion and Regeneration of Hematopoietic Stem Cells. *Nat. Med.* **2010**, *16*, 475–482. [CrossRef]
30. Santulli, G. Angiopoietin-Like Proteins: A Comprehensive Look. *Front. Endocrinol.* **2014**, *5*, 4. [CrossRef]
31. Bhardwaj, G.; Murdoch, B.; Wu, D.; Baker, D.P.; Williams, K.P.; Chadwick, K.; Ling, L.E.; Karanu, F.N.; Bhatia, M. Sonic Hedgehog Induces the Proliferation of Primitive Human Hematopoietic Cells via BMP Regulation. *Nat. Immunol.* **2001**, *2*, 172–180. [CrossRef] [PubMed]
32. Bai, T.; Li, J.; Sinclair, A.; Imren, S.; Merriam, F.; Sun, F.; O'Kelly, M.B.; Nourigat, C.; Jain, P.; Delrow, J.J.; et al. Expansion of Primitive Human Hematopoietic Stem Cells by Culture in a Zwitterionic Hydrogel. *Nat. Med.* **2019**, *25*, 1566–1575. [CrossRef]
33. Williams, D.E.; Eisenman, J.; Baird, A.; Rauch, C.; Van Ness, K.; March, C.J.; Park, L.S.; Martin, U.; Mochizukl, D.Y.; Boswell, H.S.; et al. Identification of a Ligand for the C-Kit Proto-Oncogene. *Cell* **1990**, *63*, 167–174. [CrossRef] [PubMed]
34. Martin, F.H.; Suggs, S.V.; Langley, K.E.; Lu, H.S.; Ting, J.; Okino, K.H.; Morris, C.F.; McNiece, I.K.; Jacobsen, F.W.; Mendlaz, E.A.; et al. Primary Structure and Functional Expression of Rat and Human Stem Cell Factor DNAs. *Cell* **1990**, *63*, 203–211. [CrossRef]
35. Zsebo, K.M.; Williams, D.A.; Geissler, E.N.; Broudy, V.C.; Martin, F.H.; Atkins, H.L.; Hsu, R.-Y.; Birkett, N.C.; Okino, K.H.; Murdock, D.C.; et al. Stem Cell Factor Is Encoded at the SI Locus of the Mouse and Is the Ligand for the C-Kit Tyrosine Kinase Receptor. *Cell* **1990**, *63*, 213–224. [CrossRef] [PubMed]
36. Bernstein, I.D.; Andrews, R.G.; Zsebo, K.M. Recombinant Human Stem Cell Factor Enhances the Formation of Colonies by $CD34^+$ and $CD34^+$lin- Cells, and the Generation of Colony-Forming Cell Progeny From $CD34^+$lin- Cells Cultured With Interleukin-3, Granulocyte Colony-Stimulating Factor, or Granulocyte-Macrophage Colony-Stimulating Factor. *Blood* **1991**, *77*, 2316–2321. [CrossRef] [PubMed]
37. Duarte, R.F.; Frank, D.A. The Synergy Between Stem Cell Factor (SCF) and Granulocyte Colony-Stimulating Factor (G-CSF): Molecular Basis and Clinical Relevance. *Leuk. Lymphoma* **2002**, *43*, 1179–1187. [CrossRef]
38. Ding, L.; Saunders, T.L.; Enikolopov, G.; Morrison, S.J. Endothelial and Perivascular Cells Maintain Haematopoietic Stem Cells. *Nature* **2012**, *481*, 457–462. [CrossRef]
39. Yamazaki, S.; Iwama, A.; Takayanagi, S.; Morita, Y.; Eto, K.; Ema, H.; Nakauchi, H. Cytokine Signals Modulated via Lipid Rafts Mimic Niche Signals and Induce Hibernation in Hematopoietic Stem Cells. *EMBO J.* **2006**, *25*, 3515–3523. [CrossRef]
40. Miyamoto, K.; Araki, K.Y.; Naka, K.; Arai, F.; Takubo, K.; Yamazaki, S.; Matsuoka, S.; Miyamoto, T.; Ito, K.; Ohmura, M.; et al. Foxo3a Is Essential for Maintenance of the Hematopoietic Stem Cell Pool. *Cell Stem Cell* **2007**, *1*, 101–112. [CrossRef]

41. Rönnstrand, L. Signal Transduction via the Stem Cell Factor Receptor/c-Kit. *CMLS Cell. Mol. Life Sci.* **2004**, *61*, 2535–2548. [CrossRef] [PubMed]
42. Bartley, T. Identification and Cloning of a Megakaryocyte Growth and Development Factor That Is a Ligand for the Cytokine Receptor Mpl. *Cell* **1994**, *77*, 1117–1124. [CrossRef] [PubMed]
43. de Sauvage, F.J.; Hass, P.E.; Spencer, S.D.; Malloy, B.E.; Gurney, A.L.; Spencer, S.A.; Darbonne, W.C.; Henzel, W.J.; Wong, S.C.; Kuang, W.-J.; et al. Stimulation of Megakaryocytopoiesis and Thrombopoiesis by the C-Mpl Ligand. *Nature* **1994**, *369*, 533–538. [CrossRef]
44. Kaushansky, K.; Lok, S.; Holly, R.D.; Broudy, V.C.; Lin, N.; Bailey, M.C.; Forstrom, J.W.; Buddle, M.M.; Oort, P.J.; Hagen, F.S.; et al. Promotion of Megakaryocyte Progenitor Expansion and Differentiation by the C-Mpl Ligand Thrombopoietin. *Nature* **1994**, *369*, 568–571. [CrossRef] [PubMed]
45. Lok, S.; Kaushansky, K.; Holly, R.D.; Kuijper, J.L.; Lofton-Day, C.E.; Oort, P.J.; Grant, F.J.; Heipel, M.D.; Burkhead, S.K.; Kramer, J.M.; et al. Cloning and Expression of Murine Thrombopoietin CDNA and Stimulation of Platelet Production in Vivo. *Nature* **1994**, *369*, 565–568. [CrossRef]
46. Sohma, Y.; Akahori, H.; Seki, N.; Hori, T.; Ogami, K.; Kato, T.; Shimada, Y.; Kawamura, K.; Miyazaki, H. Molecular Cloning and Chromosomal Localization of the Human Thrombopoietin Gene. *FEBS Lett.* **1994**, *353*, 57–61. [CrossRef]
47. Wendling, F.; Maraskovsky, E.; Debili, N.; Florindo, C.; Teepe, M.; Titeux, M.; Methia, N.; Breton-Gorius, J.; Cosman, D.; Vainchenker, W. C-Mpl Ligand Is a Humoral Regulator of Megakaryocytopoiesis. *Nature* **1994**, *369*, 571–574. [CrossRef]
48. Qian, H.; Buza-Vidas, N.; Hyland, C.D.; Jensen, C.T.; Antonchuk, J.; Månsson, R.; Thoren, L.A.; Ekblom, M.; Alexander, W.S.; Jacobsen, S.E.W. Critical Role of Thrombopoietin in Maintaining Adult Quiescent Hematopoietic Stem Cells. *Cell Stem Cell* **2007**, *1*, 671–684. [CrossRef]
49. Yoshihara, H.; Arai, F.; Hosokawa, K.; Hagiwara, T.; Takubo, K.; Nakamura, Y.; Gomei, Y.; Iwasaki, H.; Matsuoka, S.; Miyamoto, K.; et al. Thrombopoietin/MPL Signaling Regulates Hematopoietic Stem Cell Quiescence and Interaction with the Osteoblastic Niche. *Cell Stem Cell* **2007**, *1*, 685–697. [CrossRef]
50. Fox, N.; Priestley, G.; Papayannopoulou, T.; Kaushansky, K. Thrombopoietin Expands Hematopoietic Stem Cells after Transplantation. *J. Clin. Investig.* **2002**, *110*, 389–394. [CrossRef]
51. Kovtonyuk, L.V.; Manz, M.G.; Takizawa, H. Enhanced Thrombopoietin but Not G-CSF Receptor Stimulation Induces Self-Renewing Hematopoietic Stem Cell Divisions in Vivo. *Blood* **2016**, *127*, 3175–3179. [CrossRef] [PubMed]
52. Kimura, S.; Roberts, A.W.; Metcalf, D.; Alexander, W.S. Hematopoietic Stem Cell Deficiencies in Mice Lacking C-Mpl, the Receptor for Thrombopoietin. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1195–1200. [CrossRef] [PubMed]
53. Miyakawa, Y.; Drachman, J.G.; Gallis, B.; Kaushansky, A.; Kaushansky, K. A Structure-Function Analysis of Serine/Threonine Phosphorylation of the Thrombopoietin Receptor, c-Mpl. *J. Biol. Chem.* **2000**, *275*, 32214–32219. [CrossRef] [PubMed]
54. Miyakawa, Y.; Oda, A.; Druker, B.; Kato, T.; Miyazaki, H.; Handa, M.; Ikeda, Y. Recombinant Thrombopoietin Induces Rapid Protein Tyrosine Phosphorylation of Janus Kinase 2 and Shc in Human Blood Platelets. *Blood* **1995**, *86*, 23–27. [CrossRef]
55. Miyakawa, Y.; Oda, A.; Druker, B.; Miyazaki, H.; Handa, M.; Ohashi, H.; Ikeda, Y. Thrombopoietin Induces Tyrosine Phosphorylation of Stat3 and Stat5 in Human Blood Platelets. *Blood* **1996**, *87*, 439–446. [CrossRef]
56. Yamada, M.; Komatsu, N.; Okada, K.; Kato, T.; Miyazaki, H.; Miura, Y. Thrombopoietin Induces Tyrosine Phosphorylation and Activation of Mitogen-Activated Protein Kinases in a Human Thrombopoietin-Dependent Cell Line. *Biochem. Biophys. Res. Commun.* **1995**, *217*, 230–237. [CrossRef]
57. Lyman, S.D.; James, L.; Bos, T.V.; de Vries, P.; Brasel, K.; Gliniak, B.; Hollingsworth, L.T.; Picha, K.S.; McKenna, H.J.; Splett, R.R.; et al. Molecular Cloning of a Ligand for the Flt3flk-2 Tyrosine Kinase Receptor: A Proliferative Factor for Primitive Hematopoietic Cells. *Cell* **1993**, *75*, 1157–1167. [CrossRef]
58. Hannum, C.; Culpepper, J.; Campbell, D.; McClanahan, T.; Zurawski, S.; Kastelein, R.; Bazan, J.F.; Hudak, S.; Wagner, J.; Mattson, J.; et al. Ligand for FLT3/FLK2 Receptor Tyrosine Kinase Regulates Growth of Haematopoietic Stem Cells and Is Encoded by Variant RNAs. *Nature* **1994**, *368*, 643–648. [CrossRef]
59. Masson, K.; Rönnstrand, L. Oncogenic Signaling from the Hematopoietic Growth Factor Receptors C-Kit and Flt3. *Cell. Signal.* **2009**, *21*, 1717–1726. [CrossRef]
60. Levis, M.; Small, D. FLT3: ITDoes Matter in Leukemia. *Leukemia* **2003**, *17*, 1738–1752. [CrossRef]
61. Tenen, D.G. Disruption of Differentiation in Human Cancer: AML Shows the Way. *Nat. Rev. Cancer* **2003**, *3*, 89–101. [CrossRef] [PubMed]
62. Broxmeyer, H.E.; Lu, L.; Cooper, S.; Ruggieri, L.; Li, Z.H.; Lyman, S.D. Flt3 Ligand Stimulates/Costimulates the Growth of Myeloid Stem/Progenitor Cells. *Exp. Hematol.* **1995**, *23*, 1121–1129. [PubMed]
63. Yoo, E.-S.; Ryu, K.-H.; Park, H.-Y.; Seong, C.-M.; Chung, W.-S.; Kim, S.-C.; Choi, Y.-M.; Hahn, M.-J.; Woo, S.-Y.; Seoh, J.-Y. Myeloid Differentiation of Human Cord Blood $CD34^+$ Cells during Ex Vivo Expansion Using Thrombopoietin, Flt3-Ligand and/or Granulocyte-Colony Stimulating Factor. *Br. J. Haematol.* **1999**, *105*, 1034–1040. [CrossRef]
64. Hunter, C.A.; Jones, S.A. IL-6 as a Keystone Cytokine in Health and Disease. *Nat. Immunol.* **2015**, *16*, 448–457. [CrossRef] [PubMed]
65. Heinrich, P.C.; Behrmann, I.; Haan, S.; Hermanns, H.M.; MÜller-Newen, G.; Schaper, F. Principles of Interleukin (IL)-6-Type Cytokine Signalling and Its Regulation. *Biochem. J.* **2003**, *374*, 1–20. [CrossRef]

66. Johnson, D.E.; O'Keefe, R.A.; Grandis, J.R. Targeting the IL-6/JAK/STAT3 Signalling Axis in Cancer. *Nat. Rev. Clin. Oncol.* **2018**, *15*, 234–248. [CrossRef]
67. Skiniotis, G.; Boulanger, M.J.; Garcia, K.C.; Walz, T. Signaling Conformations of the Tall Cytokine Receptor Gp130 When in Complex with IL-6 and IL-6 Receptor. *Nat. Struct. Mol. Biol.* **2005**, *12*, 545–551. [CrossRef]
68. Butler, J.M.; Nolan, D.J.; Vertes, E.L.; Varnum-Finney, B.; Kobayashi, H.; Hooper, A.T.; Seandel, M.; Shido, K.; White, I.A.; Kobayashi, M.; et al. Endothelial Cells Are Essential for the Self-Renewal and Repopulation of Notch-Dependent Hematopoietic Stem Cells. *Cell Stem Cell* **2010**, *6*, 251–264. [CrossRef]
69. Poulos, M.G.; Guo, P.; Kofler, N.M.; Pinho, S.; Gutkin, M.C.; Tikhonova, A.; Aifantis, I.; Frenette, P.S.; Kitajewski, J.; Rafii, S.; et al. Endothelial Jagged-1 Is Necessary for Homeostatic and Regenerative Hematopoiesis. *Cell Rep.* **2013**, *4*, 1022–1034. [CrossRef]
70. Negishi, N.; Suzuki, D.; Ito, R.; Irie, N.; Matsuo, K.; Yahata, T.; Nagano, K.; Aoki, K.; Ohya, K.; Hozumi, K.; et al. Effective Expansion of Engrafted Human Hematopoietic Stem Cells in Bone Marrow of Mice Expressing Human Jagged1. *Exp. Hematol.* **2014**, *42*, 487–494.e1. [CrossRef]
71. Delaney, C.; Varnum-Finney, B.; Aoyama, K.; Brashem-Stein, C.; Bernstein, I.D. Dose-Dependent Effects of the Notch Ligand Delta1 on Ex Vivo Differentiation and in Vivo Marrow Repopulating Ability of Cord Blood Cells. *Blood* **2005**, *106*, 2693–2699. [CrossRef] [PubMed]
72. Delaney, C.; Heimfeld, S.; Brashem-Stein, C.; Voorhies, H.; Manger, R.L.; Bernstein, I.D. Notch-Mediated Expansion of Human Cord Blood Progenitor Cells Capable of Rapid Myeloid Reconstitution. *Nat. Med.* **2010**, *16*, 232–236. [CrossRef] [PubMed]
73. Ohishi, K.; Varnum-Finney, B.; Bernstein, I.D. Delta-1 Enhances Marrow and Thymus Repopulating Ability of Human $CD34^+CD38^-$ Cord Blood Cells. *J. Clin. Investig.* **2002**, *110*, 1165–1174. [CrossRef] [PubMed]
74. de Lima, M.; McNiece, I.; Robinson, S.N.; Munsell, M.; Eapen, M.; Horowitz, M.; Alousi, A.; Saliba, R.; McMannis, J.D.; Kaur, I.; et al. Cord-Blood Engraftment with Ex Vivo Mesenchymal-Cell Coculture. *N. Engl. J. Med.* **2012**, *367*, 2305–2315. [CrossRef]
75. Lewis, N.S.; Lewis, E.E.; Mullin, M.; Wheadon, H.; Dalby, M.J.; Berry, C.C. Magnetically Levitated Mesenchymal Stem Cell Spheroids Cultured with a Collagen Gel Maintain Phenotype and Quiescence. *J. Tissue Eng.* **2017**, *8*, 204173141770442. [CrossRef]
76. Khan, A.O.; Rodriguez-Romera, A.; Reyat, J.S.; Olijnik, A.-A.; Colombo, M.; Wang, G.; Wen, W.X.; Sousos, N.; Murphy, L.C.; Grygielska, B.; et al. Human Bone Marrow Organoids for Disease Modeling, Discovery, and Validation of Therapeutic Targets in Hematologic Malignancies. *Cancer Discov.* **2023**, *13*, 364–385. [CrossRef]
77. Peled, T.; Landau, E.; Mandel, J.; Glukhman, E.; Goudsmid, N.R.; Nagler, A.; Fibach, E. Linear Polyamine Copper Chelator Tetraethylenepentamine Augments Long-Term Ex Vivo Expansion of Cord Blood-Derived $CD34^+$ Cells and Increases Their Engraftment Potential in NOD/SCID Mice. *Exp. Hematol.* **2004**, *32*, 547–555. [CrossRef]
78. Peled, T.; Mandel, J.; Goudsmid, R.N.; Landor, C.; Hasson, N.; Harati, D.; Austin, M.; Hasson, A.; Fibach, E.; Shpall, E.J.; et al. Pre-Clinical Development of Cord Blood-Derived Progenitor Cell Graft Expanded Ex Vivo with Cytokines and the Polyamine Copper Chelator Tetraethylenepentamine. *Cytotherapy* **2004**, *6*, 344–355. [CrossRef]
79. Peled, T.; Landau, E.; Prus, E.; Treves, A.J.; Fibach, E. Cellular Copper Content Modulates Differentiation and Self-Renewal in Cultures of Cord Blood-Derived $CD34^+$ Cells: Copper Modulates Differentiation/Self-Renewal of CD34 Cells. *Br. J. Haematol.* **2002**, *116*, 655–661. [CrossRef]
80. Peled, T.; Glukhman, E.; Hasson, N.; Adi, S.; Assor, H.; Yudin, D.; Landor, C.; Mandel, J.; Landau, E.; Prus, E.; et al. Chelatable Cellular Copper Modulates Differentiation and Self-Renewal of Cord Blood–Derived Hematopoietic Progenitor Cells. *Exp. Hematol.* **2005**, *33*, 1092–1100. [CrossRef]
81. de Lima, M.; McMannis, J.; Gee, A.; Komanduri, K.; Couriel, D.; Andersson, B.S.; Hosing, C.; Khouri, I.; Jones, R.; Champlin, R.; et al. Transplantation of Ex Vivo Expanded Cord Blood Cells Using the Copper Chelator Tetraethylenepentamine: A Phase I/II Clinical Trial. *Bone Marrow Transpl.* **2008**, *41*, 771–778. [CrossRef] [PubMed]
82. Stiff, P.J.; Montesinos, P.; Peled, T.; Landau, E.; Goudsmid, N.R.; Mandel, J.; Hasson, N.; Olesinski, E.; Glukhman, E.; Snyder, D.A.; et al. Cohort-Controlled Comparison of Umbilical Cord Blood Transplantation Using Carlecortemcel-L, a Single Progenitor–Enriched Cord Blood, to Double Cord Blood Unit Transplantation. *Biol. Blood Marrow Transplant.* **2018**, *24*, 1463–1470. [CrossRef] [PubMed]
83. Wagner, J.E.; Brunstein, C.G.; Boitano, A.E.; DeFor, T.E.; McKenna, D.; Sumstad, D.; Blazar, B.R.; Tolar, J.; Le, C.; Jones, J.; et al. Phase I/II Trial of StemRegenin-1 Expanded Umbilical Cord Blood Hematopoietic Stem Cells Supports Testing as a Stand-Alone Graft. *Cell Stem Cell* **2016**, *18*, 144–155. [CrossRef] [PubMed]
84. Peled, T.; Shoham, H.; Aschengrau, D.; Yackoubov, D.; Frei, G.; Rosenheimer G, N.; Lerrer, B.; Cohen, H.Y.; Nagler, A.; Fibach, E.; et al. Nicotinamide, a SIRT1 Inhibitor, Inhibits Differentiation and Facilitates Expansion of Hematopoietic Progenitor Cells with Enhanced Bone Marrow Homing and Engraftment. *Exp. Hematol.* **2012**, *40*, 342–355.e1. [CrossRef] [PubMed]
85. Horwitz, M.E.; Chao, N.J.; Rizzieri, D.A.; Long, G.D.; Sullivan, K.M.; Gasparetto, C.; Chute, J.P.; Morris, A.; McDonald, C.; Waters-Pick, B.; et al. Umbilical Cord Blood Expansion with Nicotinamide Provides Long-Term Multilineage Engraftment. *J. Clin. Investig.* **2014**, *124*, 3121–3128. [CrossRef]
86. Horwitz, M.E.; Wease, S.; Blackwell, B.; Valcarcel, D.; Frassoni, F.; Boelens, J.J.; Nierkens, S.; Jagasia, M.; Wagner, J.E.; Kuball, J.; et al. Phase I/II Study of Stem-Cell Transplantation Using a Single Cord Blood Unit Expanded Ex Vivo With Nicotinamide. *JCO* **2019**, *37*, 367–374. [CrossRef]

87. Goessling, W.; North, T.E.; Loewer, S.; Lord, A.M.; Lee, S.; Stoick-Cooper, C.L.; Weidinger, G.; Puder, M.; Daley, G.Q.; Moon, R.T.; et al. Genetic Interaction of PGE2 and Wnt Signaling Regulates Developmental Specification of Stem Cells and Regeneration. *Cell* **2009**, *136*, 1136–1147. [CrossRef]
88. Ikushima, Y.M.; Arai, F.; Hosokawa, K.; Toyama, H.; Takubo, K.; Furuyashiki, T.; Narumiya, S.; Suda, T. Prostaglandin E2 Regulates Murine Hematopoietic Stem/Progenitor Cells Directly via EP4 Receptor and Indirectly through Mesenchymal Progenitor Cells. *Blood* **2013**, *121*, 1995–2007. [CrossRef]
89. North, T.E.; Goessling, W.; Walkley, C.R.; Lengerke, C.; Kopani, K.R.; Lord, A.M.; Weber, G.J.; Bowman, T.V.; Jang, I.-H.; Grosser, T.; et al. Prostaglandin E2 Regulates Vertebrate Haematopoietic Stem Cell Homeostasis. *Nature* **2007**, *447*, 1007–1011. [CrossRef]
90. Goessling, W.; Allen, R.S.; Guan, X.; Jin, P.; Uchida, N.; Dovey, M.; Harris, J.M.; Metzger, M.E.; Bonifacino, A.C.; Stroncek, D.; et al. Prostaglandin E2 Enhances Human Cord Blood Stem Cell Xenotransplants and Shows Long-Term Safety in Preclinical Nonhuman Primate Transplant Models. *Cell Stem Cell* **2011**, *8*, 445–458. [CrossRef]
91. Cutler, C.; Multani, P.; Robbins, D.; Kim, H.T.; Le, T.; Hoggatt, J.; Pelus, L.M.; Desponts, C.; Chen, Y.-B.; Rezner, B.; et al. Prostaglandin-Modulated Umbilical Cord Blood Hematopoietic Stem Cell Transplantation. *Blood* **2013**, *122*, 3074–3081. [CrossRef] [PubMed]
92. Zhang, Y.; Desai, A.; Yang, S.Y.; Bae, K.B.; Antczak, M.I.; Fink, S.P.; Tiwari, S.; Willis, J.E.; Williams, N.S.; Dawson, D.M.; et al. Inhibition of the Prostaglandin-Degrading Enzyme 15-PGDH Potentiates Tissue Regeneration. *Science* **2015**, *348*, aaa2340. [CrossRef] [PubMed]
93. Desai, A.; Zhang, Y.; Park, Y.; Dawson, D.M.; Larusch, G.A.; Kasturi, L.; Wald, D.; Ready, J.M.; Gerson, S.L.; Markowitz, S.D. A Second-Generation 15-PGDH Inhibitor Promotes Bone Marrow Transplant Recovery Independently of Age, Transplant Dose and Granulocyte Colony-Stimulating Factor Support. *Haematologica* **2018**, *103*, 1054–1064. [CrossRef] [PubMed]
94. Fares, I.; Chagraoui, J.; Gareau, Y.; Gingras, S.; Ruel, R.; Mayotte, N.; Csaszar, E.; Knapp, D.J.H.F.; Miller, P.; Ngom, M.; et al. Pyrimidoindole Derivatives Are Agonists of Human Hematopoietic Stem Cell Self-Renewal. *Science* **2014**, *345*, 1509–1512. [CrossRef]
95. Subramaniam, A.; Žemaitis, K.; Talkhoncheh, M.S.; Yudovich, D.; Bäckström, A.; Debnath, S.; Chen, J.; Jain, M.V.; Galeev, R.; Gaetani, M.; et al. Lysine-Specific Demethylase 1A Restricts Ex Vivo Propagation of Human HSCs and Is a Target of UM171. *Blood* **2020**, *136*, 2151–2161. [CrossRef]
96. Cohen, S.; Roy, J.; Lachance, S.; Delisle, J.-S.; Marinier, A.; Busque, L.; Roy, D.-C.; Barabé, F.; Ahmad, I.; Bambace, N.; et al. Hematopoietic Stem Cell Transplantation Using Single UM171-Expanded Cord Blood: A Single-Arm, Phase 1–2 Safety and Feasibility Study. *Lancet Haematol.* **2020**, *7*, e134–e145. [CrossRef]
97. Feng, Y.; Xie, X.-Y.; Yang, Y.-Q.; Sun, Y.-T.; Ma, W.-H.; Zhou, P.-J.; Li, Z.-Y.; Liu, H.-Q.; Wang, Y.-F.; Huang, Y.-S. Synthesis and Evaluation of Pyrimidoindole Analogs in Umbilical Cord Blood Ex Vivo Expansion. *Eur. J. Med. Chem.* **2019**, *174*, 181–197. [CrossRef]
98. Nishino, T.; Miyaji, K.; Ishiwata, N.; Arai, K.; Yui, M.; Asai, Y.; Nakauchi, H.; Iwama, A. Ex Vivo Expansion of Human Hematopoietic Stem Cells by a Small-Molecule Agonist of c-MPL. *Exp. Hematol.* **2009**, *37*, 1364–1377.e4. [CrossRef]
99. Sun, H.; Tsai, Y.; Nowak, I.; Liesveld, J.; Chen, Y. Eltrombopag, a Thrombopoietin Receptor Agonist, Enhances Human Umbilical Cord Blood Hematopoietic Stem/Primitive Progenitor Cell Expansion and Promotes Multi-Lineage Hematopoiesis. *Stem Cell Res.* **2012**, *9*, 77–86. [CrossRef]
100. Kao, Y.-R.; Chen, J.; Narayanagari, S.-R.; Todorova, T.I.; Aivalioti, M.M.; Ferreira, M.; Ramos, P.M.; Pallaud, C.; Mantzaris, I.; Shastri, A.; et al. Thrombopoietin Receptor–Independent Stimulation of Hematopoietic Stem Cells by Eltrombopag. *Sci. Transl. Med.* **2018**, *10*, eaas9563. [CrossRef]
101. Geest, C.R.; Coffer, P.J. MAPK Signaling Pathways in the Regulation of Hematopoiesis. *J. Leukoc. Biol.* **2009**, *86*, 237–250. [CrossRef] [PubMed]
102. Zou, J.; Zou, P.; Wang, J.; Li, L.; Wang, Y.; Zhou, D.; Liu, L. Inhibition of P38 MAPK Activity Promotes Ex Vivo Expansion of Human Cord Blood Hematopoietic Stem Cells. *Ann. Hematol.* **2012**, *91*, 813–823. [CrossRef] [PubMed]
103. Bari, S.; Zhong, Q.; Fan, X.; Poon, Z.; Lim, A.S.T.; Lim, T.H.; Dighe, N.; Li, S.; Chiu, G.N.C.; Chai, C.L.L.; et al. Ex Vivo Expansion of $CD34^+CD90^+CD49f^+$ Hematopoietic Stem and Progenitor Cells from Non-Enriched Umbilical Cord Blood with Azole Compounds. *Stem Cells Transl. Med.* **2018**, *7*, 376–393. [CrossRef] [PubMed]
104. Xiao, X.; Lai, W.; Xie, H.; Liu, Y.; Guo, W.; Liu, Y.; Li, Y.; Li, Y.; Zhang, J.; Chen, W.; et al. Targeting JNK Pathway Promotes Human Hematopoietic Stem Cell Expansion. *Cell Discov.* **2019**, *5*, 2. [CrossRef]
105. Zhang, T.; Inesta-Vaquera, F.; Niepel, M.; Zhang, J.; Ficarro, S.B.; Machleidt, T.; Xie, T.; Marto, J.A.; Kim, N.; Sim, T.; et al. Discovery of Potent and Selective Covalent Inhibitors of JNK. *Chem. Biol.* **2012**, *19*, 140–154. [CrossRef]
106. Yu, H.; Yuan, Y.; Shen, H.; Cheng, T. Hematopoietic Stem Cell Exhaustion Impacted by P18INK4C and P21Cip1/Waf1 in Opposite Manners. *Blood* **2006**, *107*, 1200–1206. [CrossRef]
107. Yuan, Y.; Shen, H.; Franklin, D.S.; Scadden, D.T.; Cheng, T. In Vivo Self-Renewing Divisions of Haematopoietic Stem Cells Are Increased in the Absence of the Early G1-Phase Inhibitor, P18INK4C. *Nat. Cell Biol.* **2004**, *6*, 436–442. [CrossRef]
108. Gao, Y.; Yang, P.; Shen, H.; Yu, H.; Song, X.; Zhang, L.; Zhang, P.; Cheng, H.; Xie, Z.; Hao, S.; et al. Small-Molecule Inhibitors Targeting INK4 Protein P18INK4C Enhance Ex Vivo Expansion of Haematopoietic Stem Cells. *Nat. Commun.* **2015**, *6*, 6328. [CrossRef]

109. Xie, X.-Q.; Yang, P.; Zhang, Y.; Zhang, P.; Wang, L.; Ding, Y.; Yang, M.; Tong, Q.; Cheng, H.; Ji, Q.; et al. Discovery of Novel INK4C Small-Molecule Inhibitors to Promote Human and Murine Hematopoietic Stem Cell Ex Vivo Expansion. *Sci. Rep.* **2015**, *5*, 18115. [CrossRef]
110. Li, Y.; Zhang, W.; Zhang, Y.; Ding, Y.; Yang, M.; He, M.; Liu, X.; Gu, J.; Xu, S.; Feng, Z.; et al. Enhanced Self-Renewal of Human Long-Term Hematopoietic Stem Cells by a Sulfamoyl Benzoate Derivative Targeting P18INK4C. *Blood Adv.* **2021**, *5*, 3362–3372. [CrossRef]
111. Bug, G.; Gül, H.; Schwarz, K.; Pfeifer, H.; Kampfmann, M.; Zheng, X.; Beissert, T.; Boehrer, S.; Hoelzer, D.; Ottmann, O.G.; et al. Valproic Acid Stimulates Proliferation and Self-Renewal of Hematopoietic Stem Cells. *Cancer Res.* **2005**, *65*, 2537–2541. [CrossRef] [PubMed]
112. De Felice, L.; Tatarelli, C.; Mascolo, M.G.; Gregorj, C.; Agostini, F.; Fiorini, R.; Gelmetti, V.; Pascale, S.; Padula, F.; Petrucci, M.T.; et al. Histone Deacetylase Inhibitor Valproic Acid Enhances the Cytokine-Induced Expansion of Human Hematopoietic Stem Cells. *Cancer Res.* **2005**, *65*, 1505–1513. [CrossRef] [PubMed]
113. Papa, L.; Zimran, E.; Djedaini, M.; Ge, Y.; Ozbek, U.; Sebra, R.; Sealfon, S.C.; Hoffman, R. Ex Vivo Human HSC Expansion Requires Coordination of Cellular Reprogramming with Mitochondrial Remodeling and P53 Activation. *Blood Adv.* **2018**, *2*, 2766–2779. [CrossRef] [PubMed]
114. Zimran, E.; Papa, L.; Djedaini, M.; Patel, A.; Iancu-Rubin, C.; Hoffman, R. Expansion and Preservation of the Functional Activity of Adult Hematopoietic Stem Cells Cultured Ex Vivo with a Histone Deacetylase Inhibitor. *Stem Cells Transl. Med.* **2020**, *9*, 531–542. [CrossRef]
115. Chaurasia, P.; Gajzer, D.C.; Schaniel, C.; D'Souza, S.; Hoffman, R. Epigenetic Reprogramming Induces the Expansion of Cord Blood Stem Cells. *J. Clin. Investig.* **2014**, *124*, 2378–2395. [CrossRef]
116. Seet, L.-F.; Teng, E.; Lai, Y.-S.; Laning, J.; Kraus, M.; Wnendt, S.; Merchav, S.; Chan, S.L. Valproic Acid Enhances the Engraftability of Human Umbilical Cord Blood Hematopoietic Stem Cells Expanded under Serum-Free Conditions*. *Eur. J. Haematol.* **2009**, *82*, 124–132. [CrossRef]
117. Nishino, T.; Wang, C.; Mochizuki-Kashio, M.; Osawa, M.; Nakauchi, H.; Iwama, A. Ex Vivo Expansion of Human Hematopoietic Stem Cells by Garcinol, a Potent Inhibitor of Histone Acetyltransferase. *PLoS ONE* **2011**, *6*, e24298. [CrossRef]
118. Araki, H.; Mahmud, N.; Milhem, M.; Nunez, R.; Xu, M.; Beam, C.A.; Hoffman, R. Expansion of Human Umbilical Cord Blood SCID-Repopulating Cells Using Chromatin-Modifying Agents. *Exp. Hematol.* **2006**, *34*, 140–149. [CrossRef] [PubMed]
119. Saraf, S.; Araki, H.; Petro, B.; Park, Y.; Taioli, S.; Yoshinaga, K.G.; Koca, E.; Rondelli, D.; Mahmud, N. Ex Vivo Expansion of Human Mobilized Peripheral Blood Stem Cells Using Epigenetic Modifiers: Epigenetic Modifiers and PBSC Expansion. *Transfusion* **2015**, *55*, 864–874. [CrossRef]
120. Araki, H.; Yoshinaga, K.; Boccuni, P.; Zhao, Y.; Hoffman, R.; Mahmud, N. Chromatin-Modifying Agents Permit Human Hematopoietic Stem Cells to Undergo Multiple Cell Divisions While Retaining Their Repopulating Potential. *Blood* **2007**, *109*, 3570–3578. [CrossRef]
121. Hua, P.; Hester, J.; Adigbli, G.; Li, R.; Psaila, B.; Roy, A.; Bataille, C.J.R.; Wynne, G.M.; Jackson, T.; Milne, T.A.; et al. The BET Inhibitor CPI203 Promotes Ex Vivo Expansion of Cord Blood Long-Term Repopulating HSCs and Megakaryocytes. *Blood* **2020**, *136*, 2410–2415. [CrossRef] [PubMed]
122. Trowbridge, J.J.; Xenocostas, A.; Moon, R.T.; Bhatia, M. Glycogen Synthase Kinase-3 Is an in Vivo Regulator of Hematopoietic Stem Cell Repopulation. *Nat. Med.* **2006**, *12*, 89–98. [CrossRef] [PubMed]
123. Perry, J.M.; He, X.C.; Sugimura, R.; Grindley, J.C.; Haug, J.S.; Ding, S.; Li, L. Cooperation between Both Wnt/β-Catenin and PTEN/PI3K/Akt Signaling Promotes Primitive Hematopoietic Stem Cell Self-Renewal and Expansion. *Genes Dev.* **2011**, *25*, 1928–1942. [CrossRef] [PubMed]
124. Ko, K.-H.; Holmes, T.; Palladinetti, P.; Song, E.; Nordon, R.; O'Brien, T.A.; Dolnikov, A. GSK-3β Inhibition Promotes Engraftment of Ex Vivo-Expanded Hematopoietic Stem Cells and Modulates Gene Expression. *Stem Cells* **2011**, *29*, 108–118. [CrossRef] [PubMed]
125. Huang, J.; Nguyen-McCarty, M.; Hexner, E.O.; Danet-Desnoyers, G.; Klein, P.S. Maintenance of Hematopoietic Stem Cells through Regulation of Wnt and MTOR Pathways. *Nat. Med.* **2012**, *18*, 1778–1785. [CrossRef]
126. Sakurai, M.; Ishitsuka, K.; Ito, R.; Wilkinson, A.C.; Kimura, T.; Mizutani, E.; Nishikii, H.; Sudo, K.; Becker, H.J.; Takemoto, H.; et al. Chemically Defined Cytokine-Free Expansion of Human Haematopoietic Stem Cells. *Nature* **2023**, *615*, 127–133. [CrossRef]
127. Nakeff, A.; Maat, B. Separation of Megakaryocytes From Mouse Bone Marrow by Velocity Sedimentation. *Blood* **1974**, *43*, 591–595. [CrossRef]
128. George, J.N. Platelets. *Lancet* **2000**, *355*, 1531–1539. [CrossRef]
129. McArthur, K.; Chappaz, S.; Kile, B.T. Apoptosis in Megakaryocytes and Platelets: The Life and Death of a Lineage. *Blood* **2018**, *131*, 605–610. [CrossRef]
130. Stroncek, D.F.; Rebulla, P. Platelet Transfusions. *Lancet* **2007**, *370*, 427–438. [CrossRef]
131. Squires, J.E. Indications for Platelet Transfusion in Patients with Thrombocytopenia. *Blood Transfus.* **2015**, *13*, 221–226. [CrossRef] [PubMed]
132. Sanjuan-Pla, A.; Macaulay, I.C.; Jensen, C.T.; Woll, P.S.; Luis, T.C.; Mead, A.; Moore, S.; Carella, C.; Matsuoka, S.; Jones, T.B.; et al. Platelet-Biased Stem Cells Reside at the Apex of the Haematopoietic Stem-Cell Hierarchy. *Nature* **2013**, *502*, 232–236. [CrossRef] [PubMed]

133. Seita, J.; Weissman, I.L. Hematopoietic Stem Cell: Self-renewal versus Differentiation. *WIREs Mech. Dis.* **2010**, *2*, 640–653. [CrossRef] [PubMed]
134. Choi, E.; Nichol, J.; Hokom, M.; Hornkohl, A.; Hunt, P. Platelets Generated in Vitro from Proplatelet-Displaying Human Megakaryocytes Are Functional. *Blood* **1995**, *85*, 402–413. [CrossRef] [PubMed]
135. Norol, F.; Vitrat, N.; Cramer, E.; Guichard, J.; Burstein, S.A.; Vainchenker, W.; Debili, N. Effects of Cytokines on Platelet Production from Blood and Marrow CD34$^+$ Cells. *Blood* **1998**, *91*, 830–843. [CrossRef] [PubMed]
136. Proulx, C.; Dupuis, N.; St-Amour, I.; Boyer, L.; Lemieux, R. Increased Megakaryopoiesis in Cultures of CD34-Enriched Cord Blood Cells Maintained at 39 °C: Increased Megakaryopoiesis in CB Cultures. *Biotechnol. Bioeng.* **2004**, *88*, 675–680. [CrossRef]
137. Matsunaga, T.; Tanaka, I.; Kobune, M.; Kawano, Y.; Tanaka, M.; Kuribayashi, K.; Iyama, S.; Sato, T.; Sato, Y.; Takimoto, R.; et al. Ex Vivo Large-Scale Generation of Human Platelets from Cord Blood CD34$^+$ Cells. *Stem Cells* **2006**, *24*, 2877–2887. [CrossRef]
138. Guan, X.; Qin, M.; Zhang, Y.; Wang, Y.; Shen, B.; Ren, Z.; Ding, X.; Dai, W.; Jiang, Y. Safety and Efficacy of Megakaryocytes Induced from Hematopoietic Stem Cells in Murine and Nonhuman Primate Models. *Stem Cells Transl. Med.* **2017**, *6*, 897–909. [CrossRef]
139. Yang, Y.; Liu, C.; Lei, X.; Wang, H.; Su, P.; Ru, Y.; Ruan, X.; Duan, E.; Feng, S.; Han, M.; et al. Integrated Biophysical and Biochemical Signals Augment Megakaryopoiesis and Thrombopoiesis in a Three-Dimensional Rotary Culture System. *Stem Cells Transl. Med.* **2016**, *5*, 175–185. [CrossRef]
140. Guan, X.; Wang, L.; Wang, H.; Wang, H.; Dai, W.; Jiang, Y. Good Manufacturing Practice-Grade of Megakaryocytes Produced by a Novel Ex Vivo Culturing Platform. *Clin. Transl. Sci.* **2020**, *13*, 1115–1126. [CrossRef]
141. Orkin, S.H.; Zon, L.I. Hematopoiesis: An Evolving Paradigm for Stem Cell Biology. *Cell* **2008**, *132*, 631–644. [CrossRef]
142. Pimanda, J.E.; Gttgens, B. Gene Regulatory Networks Governing Haematopoietic Stem Cell Development and Identity. *Int. J. Dev. Biol.* **2010**, *54*, 1201–1211. [CrossRef] [PubMed]
143. Starck, J.; Cohet, N.; Gonnet, C.; Sarrazin, S.; Doubeikovskaia, Z.; Doubeikovski, A.; Verger, A.; Duterque-Coquillaud, M.; Morle, F. Functional Cross-Antagonism between Transcription Factors FLI-1 and EKLF. *Mol. Cell Biol.* **2003**, *23*, 1390–1402. [CrossRef] [PubMed]
144. Doré, L.C.; Crispino, J.D. Transcription Factor Networks in Erythroid Cell and Megakaryocyte Development. *Blood* **2011**, *118*, 231–239. [CrossRef]
145. Tijssen, M.R.; Cvejic, A.; Joshi, A.; Hannah, R.L.; Ferreira, R.; Forrai, A.; Bellissimo, D.C.; Oram, S.H.; Smethurst, P.A.; Wilson, N.K.; et al. Genome-Wide Analysis of Simultaneous GATA1/2, RUNX1, FLI1, and SCL Binding in Megakaryocytes Identifies Hematopoietic Regulators. *Dev. Cell* **2011**, *20*, 597–609. [CrossRef]
146. Klimchenko, O.; Mori, M.; DiStefano, A.; Langlois, T.; Larbret, F.; Lecluse, Y.; Feraud, O.; Vainchenker, W.; Norol, F.; Debili, N. A Common Bipotent Progenitor Generates the Erythroid and Megakaryocyte Lineages in Embryonic Stem Cell–Derived Primitive Hematopoiesis. *Blood* **2009**, *114*, 1506–1517. [CrossRef] [PubMed]
147. Wilson, N.K.; Foster, S.D.; Wang, X.; Knezevic, K.; Schütte, J.; Kaimakis, P.; Chilarska, P.M.; Kinston, S.; Ouwehand, W.H.; Dzierzak, E.; et al. Combinatorial Transcriptional Control In Blood Stem/Progenitor Cells: Genome-Wide Analysis of Ten Major Transcriptional Regulators. *Cell Stem Cell* **2010**, *7*, 532–544. [CrossRef]
148. Huang, H.; Cantor, A.B. Common Features of Megakaryocytes and Hematopoietic Stem Cells: What's the Connection? *J. Cell. Biochem.* **2009**, *107*, 857–864. [CrossRef] [PubMed]
149. Fielder, P.J.; Hass, P.; Nagel, M.; Stefanich, E.; Widmer, R.; Bennett, G.L.; Keller, G.-A.; de Sauvage, F.J.; Eaton, D. Human Platelets as a Model for the Binding and Degradation of Thrombopoietin. *Blood* **1997**, *89*, 2782–2788. [CrossRef]
150. Kuter, D.; Rosenberg, R. The Reciprocal Relationship of Thrombopoietin (c-Mpl Ligand) to Changes in the Platelet Mass during Busulfan-Induced Thrombocytopenia in the Rabbit. *Blood* **1995**, *85*, 2720–2730. [CrossRef]
151. McCarty, J.; Sprugel, K.; Fox, N.; Sabath, D.; Kaushansky, K. Murine Thrombopoietin MRNA Levels Are Modulated by Platelet Count. *Blood* **1995**, *86*, 3668–3675. [CrossRef] [PubMed]
152. Van Den Oudenrijn, S.; Bruin, M.; Folman, C.C.; Peters, M.; Faulkner, L.B.; De Haas, M.; Von Dem Borne, A.E.G.K.R. Mutations in the Thrombopoietin Receptor, Mpl, in Children with Congenital Amegakaryocytic Thrombocytopenia: C-Mpl Mutations in Amegakaryocytic Thrombocytopenia. *Br. J. Haematol.* **2000**, *110*, 441–448. [CrossRef] [PubMed]
153. Commins, S.P.; Borish, L.; Steinke, J.W. Immunologic Messenger Molecules: Cytokines, Interferons, and Chemokines. *J. Allergy Clin. Immunol.* **2010**, *125*, S53–S72. [CrossRef] [PubMed]
154. Kimura, H.; Ishibashi, T.; Shikama, Y.; Okano, A.; Akiyama, Y.; Uchida, T.; Maruyama, Y. Interleukin-1 Beta (IL-1 Beta) Induces Thrombocytosis in Mice: Possible Implication of IL-6. *Blood* **1990**, *76*, 2493–2500. [CrossRef] [PubMed]
155. Kaser, A.; Brandacher, G.; Steurer, W.; Kaser, S.; Offner, F.A.; Zoller, H.; Theurl, I.; Widder, W.; Molnar, C.; Ludwiczek, O.; et al. Interleukin-6 Stimulates Thrombopoiesis through Thrombopoietin: Role in Inflammatory Thrombocytosis. *Blood* **2001**, *98*, 2720–2725. [CrossRef]
156. Segal, G.M.; Stueve, T.; Adamson, J.W. Analysis of Murine Megakaryocyte Colony Size and Ploidy: Effects of Interleukin-3. *J. Cell. Physiol.* **1988**, *137*, 537–544. [CrossRef]
157. Yang, Y.-C.; Ciarletta, A.B.; Temple, P.A.; Chung, M.P.; Kovacic, S.; Witek-Giannotti, J.S.; Leary, A.C.; Kriz, R.; Donahue, R.E.; Wong, G.G.; et al. Human IL-3 (Multi-CSF): Identification by Expression Cloning of a Novel Hematopoietic Growth Factor Related to Murine IL-3. *Cell* **1986**, *47*, 3–10. [CrossRef]

158. Nishimura, S.; Nagasaki, M.; Kunishima, S.; Sawaguchi, A.; Sakata, A.; Sakaguchi, H.; Ohmori, T.; Manabe, I.; Italiano, J.E.; Ryu, T.; et al. IL-1α Induces Thrombopoiesis through Megakaryocyte Rupture in Response to Acute Platelet Needs. *J. Cell Biol.* **2015**, *209*, 453–466. [CrossRef]
159. Steinberg, M.H.; Forget, B.G.; Higgs, D.R.; Weatherall, D.J. *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management*, 2nd ed.; Cambridge University Press: Cambridge, UK, 2009; ISBN 978-0-521-87519-6.
160. Hirani, R.; Mondy, P. Review of Full Blood Count Reference Interval Using a Large Cohort of First-Time Plasmapheresis Blood Donors. *Pathology* **2021**, *53*, 498–502. [CrossRef]
161. Cable, C.A.; Razavi, S.A.; Roback, J.D.; Murphy, D.J. RBC Transfusion Strategies in the ICU: A Concise Review. *Crit. Care Med.* **2019**, *47*, 1637–1644. [CrossRef]
162. Carson, J.L.; Stanworth, S.J.; Dennis, J.A.; Trivella, M.; Roubinian, N.; Fergusson, D.A.; Triulzi, D.; Dorée, C.; Hébert, P.C. Transfusion Thresholds for Guiding Red Blood Cell Transfusion. *Cochrane Database Syst. Rev.* **2021**, *2022*. [CrossRef]
163. Neildez-Nguyen, T.M.A.; Wajcman, H.; Marden, M.C.; Bensidhoum, M.; Moncollin, V.; Giarratana, M.-C.; Kobari, L.; Thierry, D.; Douay, L. Human Erythroid Cells Produced Ex Vivo at Large Scale Differentiate into Red Blood Cells in Vivo. *Nat. Biotechnol.* **2002**, *20*, 467–472. [CrossRef] [PubMed]
164. Giarratana, M.-C.; Kobari, L.; Lapillonne, H.; Chalmers, D.; Kiger, L.; Cynober, T.; Marden, M.C.; Wajcman, H.; Douay, L. Ex Vivo Generation of Fully Mature Human Red Blood Cells from Hematopoietic Stem Cells. *Nat. Biotechnol.* **2005**, *23*, 69–74. [CrossRef] [PubMed]
165. Miharada, K.; Hiroyama, T.; Sudo, K.; Nagasawa, T.; Nakamura, Y. Efficient Enucleation of Erythroblasts Differentiated in Vitro from Hematopoietic Stem and Progenitor Cells. *Nat. Biotechnol.* **2006**, *24*, 1255–1256. [CrossRef] [PubMed]
166. Giarratana, M.-C.; Rouard, H.; Dumont, A.; Kiger, L.; Safeukui, I.; Le Pennec, P.-Y.; François, S.; Trugnan, G.; Peyrard, T.; Marie, T.; et al. Proof of Principle for Transfusion of in Vitro–Generated Red Blood Cells. *Blood* **2011**, *118*, 5071–5079. [CrossRef]
167. Zhang, Y.; Wang, C.; Wang, L.; Shen, B.; Guan, X.; Tian, J.; Ren, Z.; Ding, X.; Ma, Y.; Dai, W.; et al. Large-Scale Ex Vivo Generation of Human Red Blood Cells from Cord Blood $CD34^+$ Cells. *Stem Cells Transl. Med.* **2017**, *6*, 1698–1709. [CrossRef]
168. Hattangadi, S.M.; Wong, P.; Zhang, L.; Flygare, J.; Lodish, H.F. From Stem Cell to Red Cell: Regulation of Erythropoiesis at Multiple Levels by Multiple Proteins, RNAs, and Chromatin Modifications. *Blood* **2011**, *118*, 6258–6268. [CrossRef]
169. Caulier, A.L.; Sankaran, V.G. Molecular and Cellular Mechanisms That Regulate Human Erythropoiesis. *Blood* **2022**, *139*, 2450–2459. [CrossRef]
170. Capellera-Garcia, S.; Pulecio, J.; Dhulipala, K.; Siva, K.; Rayon-Estrada, V.; Singbrant, S.; Sommarin, M.N.E.; Walkley, C.R.; Soneji, S.; Karlsson, G.; et al. Defining the Minimal Factors Required for Erythropoiesis through Direct Lineage Conversion. *Cell Rep.* **2016**, *15*, 2550–2562. [CrossRef]
171. Sankaran, V.G.; Ghazvinian, R.; Do, R.; Thiru, P.; Vergilio, J.-A.; Beggs, A.H.; Sieff, C.A.; Orkin, S.H.; Nathan, D.G.; Lander, E.S.; et al. Exome Sequencing Identifies GATA1 Mutations Resulting in Diamond-Blackfan Anemia. *J. Clin. Investig.* **2012**, *122*, 2439–2443. [CrossRef]
172. Katsumura, K.R.; Bresnick, E.H.; the GATA Factor Mechanisms Group. The GATA Factor Revolution in Hematology. *Blood* **2017**, *129*, 2092–2102. [CrossRef] [PubMed]
173. Wakabayashi, A.; Ulirsch, J.C.; Ludwig, L.S.; Fiorini, C.; Yasuda, M.; Choudhuri, A.; McDonel, P.; Zon, L.I.; Sankaran, V.G. Insight into GATA1 Transcriptional Activity through Interrogation of *Cis* Elements Disrupted in Human Erythroid Disorders. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 4434–4439. [CrossRef] [PubMed]
174. Gutiérrez, L.; Tsukamoto, S.; Suzuki, M.; Yamamoto-Mukai, H.; Yamamoto, M.; Philipsen, S.; Ohneda, K. Ablation of Gata1 in Adult Mice Results in Aplastic Crisis, Revealing Its Essential Role in Steady-State and Stress Erythropoiesis. *Blood* **2008**, *111*, 4375–4385. [CrossRef]
175. Fujiwara, Y.; Browne, C.P.; Cunniff, K.; Goff, S.C.; Orkin, S.H. Arrested Development of Embryonic Red Cell Precursors in Mouse Embryos Lacking Transcription Factor GATA-1. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12355–12358. [CrossRef] [PubMed]
176. Crispino, J.D.; Horwitz, M.S. GATA Factor Mutations in Hematologic Disease. *Blood* **2017**, *129*, 2103–2110. [CrossRef]
177. Abdulhay, N.J.; Fiorini, C.; Verboon, J.M.; Ludwig, L.S.; Ulirsch, J.C.; Zieger, B.; Lareau, C.A.; Mi, X.; Roy, A.; Obeng, E.A.; et al. Impaired Human Hematopoiesis Due to a Cryptic Intronic GATA1 Splicing Mutation. *J. Exp. Med.* **2019**, *216*, 1050–1060. [CrossRef] [PubMed]
178. Frontelo, P.; Manwani, D.; Galdass, M.; Karsunky, H.; Lohmann, F.; Gallagher, P.G.; Bieker, J.J. Novel Role for EKLF in Megakaryocyte Lineage Commitment. *Blood* **2007**, *110*, 3871–3880. [CrossRef]
179. Bouilloux, F.; Juban, G.; Cohet, N.; Buet, D.; Guyot, B.; Vainchenker, W.; Louache, F.; Morlé, F. EKLF Restricts Megakaryocytic Differentiation at the Benefit of Erythrocytic Differentiation. *Blood* **2008**, *112*, 576–584. [CrossRef]
180. Gnanapragasam, M.N.; McGrath, K.E.; Catherman, S.; Xue, L.; Palis, J.; Bieker, J.J. EKLF/KLF1-Regulated Cell Cycle Exit Is Essential for Erythroblast Enucleation. *Blood* **2016**, *128*, 1631–1641. [CrossRef]
181. Zhou, D.; Liu, K.; Sun, C.-W.; Pawlik, K.M.; Townes, T.M. KLF1 Regulates BCL11A Expression and γ- to β-Globin Gene Switching. *Nat. Genet.* **2010**, *42*, 742–744. [CrossRef]
182. Arnaud, L.; Saison, C.; Helias, V.; Lucien, N.; Steschenko, D.; Giarratana, M.-C.; Prehu, C.; Foliguet, B.; Montout, L.; de Brevern, A.G.; et al. A Dominant Mutation in the Gene Encoding the Erythroid Transcription Factor KLF1 Causes a Congenital Dyserythropoietic Anemia. *Am. J. Hum. Genet.* **2010**, *87*, 721–727. [CrossRef] [PubMed]

183. Borg, J.; Papadopoulos, P.; Georgitsi, M.; Gutiérrez, L.; Grech, G.; Fanis, P.; Phylactides, M.; Verkerk, A.J.M.H.; van der Spek, P.J.; Scerri, C.A.; et al. Haploinsufficiency for the Erythroid Transcription Factor KLF1 Causes Hereditary Persistence of Fetal Hemoglobin. *Nat. Genet.* **2010**, *42*, 801–805. [CrossRef]
184. Magor, G.W.; Tallack, M.R.; Gillinder, K.R.; Bell, C.C.; McCallum, N.; Williams, B.; Perkins, A.C. KLF1-Null Neonates Display Hydrops Fetalis and a Deranged Erythroid Transcriptome. *Blood* **2015**, *125*, 2405–2417. [CrossRef] [PubMed]
185. Porcher, C.; Chagraoui, H.; Kristiansen, M.S. SCL/TAL1: A Multifaceted Regulator from Blood Development to Disease. *Blood* **2017**, *129*, 2051–2060. [CrossRef]
186. Sui, X.; Krantz, S.B.; Zhao, Z.J. Stem Cell Factor and Erythropoietin Inhibit Apoptosis of Human Erythroid Progenitor Cells through Different Signalling Pathways: Distinct Roles of PI3K in SCF and EPO Signalling. *Br. J. Haematol.* **2000**, *110*, 63–70. [CrossRef] [PubMed]
187. Nocka, K.; Majumder, S.; Chabot, B.; Ray, P.; Cervone, M.; Bernstein, A.; Besmer, P. Expression of C-Kit Gene Products in Known Cellular Targets of W Mutations in Normal and W Mutant Mice–Evidence for an Impaired c-Kit Kinase in Mutant Mice. *Genes Dev.* **1989**, *3*, 816–826. [CrossRef]
188. Goodman, J.W.; Hall, E.A.; Miller, K.L.; Shinpock, S.G. Interleukin 3 Promotes Erythroid Burst Formation in "Serum-Free" Cultures without Detectable Erythropoietin. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 3291–3295. [CrossRef]
189. Migliaccio, G.; Migliaccio, A.R.; Adamson, J.W. In Vitro Differentiation of Human Granulocyte/Macrophage and Erythroid Progenitors: Comparative Analysis of the Influence of Recombinant Human Erythropoietin, G-CSF, GM-CSF, and IL-3 in Serum-Supplemented and Serum-Deprived Cultures. *Blood* **1988**, *72*, 248–256. [CrossRef]
190. Lee-Huang, S. Cloning and Expression of Human Erythropoietin CDNA in Escherichia Coli. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 2708–2712. [CrossRef]
191. Jacobs, K.; Shoemaker, C.; Rudersdorf, R.; Neill, S.D.; Kaufman, R.J.; Mufson, A.; Seehra, J.; Jones, S.S.; Hewick, R.; Fritsch, E.F.; et al. Isolation and Characterization of Genomic and CDNA Clones of Human Erythropoietin. *Nature* **1985**, *313*, 806–810. [CrossRef]
192. Koury, M.J.; Bondurant, M.C. Erythropoietin Retards DNA Breakdown and Prevents Programmed Death in Erythroid Progenitor Cells. *Science* **1990**, *248*, 378–381. [CrossRef] [PubMed]
193. Broudy, V.C.; Lin, N.; Brice, M.; Nakamoto, B.; Papayannopoulou, T. Erythropoietin Receptor Characteristics on Primary Human Erythroid Cells. *Blood* **1991**, *77*, 2583–2590. [CrossRef] [PubMed]
194. Reissmann, K.R. Studies on the Mechanism of Erythropoietic Stimulation in Parabiotic Rats during Hypoxia. *Blood* **1950**, *5*, 372–380. [CrossRef] [PubMed]
195. Wu, H.; Liu, X.; Jaenisch, R.; Lodish, H.F. Generation of Committed Erythroid BFU-E and CFU-E Progenitors Does Not Require Erythropoietin or the Erythropoietin Receptor. *Cell* **1995**, *83*, 59–67. [CrossRef]
196. Zhang, Y.; Wang, L.; Dey, S.; Alnaeeli, M.; Suresh, S.; Rogers, H.; Teng, R.; Noguchi, C. Erythropoietin Action in Stress Response, Tissue Maintenance and Metabolism. *Int. J. Mol. Sci.* **2014**, *15*, 10296–10333. [CrossRef]
197. Ugo, V.; Marzac, C.; Teyssandier, I.; Larbret, F.; Lécluse, Y.; Debili, N.; Vainchenker, W.; Casadevall, N. Multiple Signaling Pathways Are Involved in Erythropoietin-Independent Differentiation of Erythroid Progenitors in Polycythemia Vera. *Exp. Hematol.* **2004**, *32*, 179–187. [CrossRef]
198. Chida, D.; Miura, O.; Yoshimura, A.; Miyajima, A. Role of Cytokine Signaling Molecules in Erythroid Differentiation of Mouse Fetal Liver Hematopoietic Cells: Functional Analysis of Signaling Molecules by Retrovirus-Mediated Expression. *Blood* **1999**, *93*, 1567–1578. [CrossRef]
199. Arcasoy, M.O.; Jiang, X. Co-Operative Signalling Mechanisms Required for Erythroid Precursor Expansion in Response to Erythropoietin and Stem Cell Factor. *Br. J. Haematol.* **2005**, *130*, 121–129. [CrossRef]
200. Notta, F.; Zandi, S.; Takayama, N.; Dobson, S.; Gan, O.I.; Wilson, G.; Kaufmann, K.B.; McLeod, J.; Laurenti, E.; Dunant, C.F.; et al. Distinct Routes of Lineage Development Reshape the Human Blood Hierarchy across Ontogeny. *Science* **2016**, *351*, aab2116. [CrossRef]
201. Grover, A.; Mancini, E.; Moore, S.; Mead, A.J.; Atkinson, D.; Rasmussen, K.D.; O'Carroll, D.; Jacobsen, S.E.W.; Nerlov, C. Erythropoietin Guides Multipotent Hematopoietic Progenitor Cells toward an Erythroid Fate. *J. Exp. Med.* **2014**, *211*, 181–188. [CrossRef]
202. Zhang, H.; Wang, S.; Liu, D.; Gao, C.; Han, Y.; Guo, X.; Qu, X.; Li, W.; Zhang, S.; Geng, J.; et al. EpoR-TdTomato-Cre Mice Enable Identification of EpoR Expression in Subsets of Tissue Macrophages and Hematopoietic Cells. *Blood* **2021**, *138*, 1986–1997. [CrossRef] [PubMed]
203. Sherwood, J.B.; Goldwasser, E. Extraction of Erythropoietin from Normal Kidneys*. *Endocrinology* **1978**, *103*, 866–870. [CrossRef] [PubMed]
204. Hammond, D.; Winnick, S. Paraneoplastic Erythrocytosis and Ectopic Erythropoietins. *Ann. N. Y. Acad. Sci.* **1974**, *230*, 219–227. [CrossRef] [PubMed]
205. Fried, W. The Liver as a Source of Extrarenal Erythropoietin Production. *Blood* **1972**, *40*, 671–677. [CrossRef] [PubMed]
206. Naughton, B.A.; Kaplan, S.M.; Roy, M.; Burdowski, A.J.; Gordon, A.S.; Piliero, S.J. Hepatic Regeneration and Erythropoietin Production in the Rat. *Science* **1977**, *196*, 301–302. [CrossRef] [PubMed]
207. Lucarelli, G.; Howard, D.; Stohlman, F. Regulation of Erythropoiesis. XV. Neonatal Erythropoiesis and the Effect of Nephrectomy *. *J. Clin. Investig.* **1964**, *43*, 2195–2203. [CrossRef]

208. Zanjani, E.D.; Poster, J.; Burlington, H.; Mann, L.I.; Wasserman, L.R. Liver as the Primary Site of Erythropoietin Formation in the Fetus. *J. Lab. Clin. Med.* **1977**, *89*, 640–644.
209. Janeway, C.A.J.; Travers, P.; Walport, M.; Shlomchik, M.J. The Components of the Immune System. In *Immunobiology: The Immune System in Health and Disease*, 5th ed.; Garland Science: New York, NY, USA, 2001.
210. Friedman, A.D. Transcriptional Control of Granulocyte and Monocyte Development. *Oncogene* **2007**, *26*, 6816–6828. [CrossRef]
211. Huber, R.; Pietsch, D.; Günther, J.; Welz, B.; Vogt, N.; Brand, K. Regulation of Monocyte Differentiation by Specific Signaling Modules and Associated Transcription Factor Networks. *Cell. Mol. Life Sci.* **2014**, *71*, 63–92. [CrossRef]
212. Lawrence, S.M.; Corriden, R.; Nizet, V. The Ontogeny of a Neutrophil: Mechanisms of Granulopoiesis and Homeostasis. *Microbiol. Mol. Biol. Rev.* **2018**, *82*, e00057-17. [CrossRef]
213. DeKoter, R.P.; Singh, H. Regulation of B Lymphocyte and Macrophage Development by Graded Expression of PU.1. *Science* **2000**, *288*, 1439–1441. [CrossRef] [PubMed]
214. Pang, S.H.M.; de Graaf, C.A.; Hilton, D.J.; Huntington, N.D.; Carotta, S.; Wu, L.; Nutt, S.L. PU.1 Is Required for the Developmental Progression of Multipotent Progenitors to Common Lymphoid Progenitors. *Front. Immunol.* **2018**, *9*, 1264. [CrossRef] [PubMed]
215. Bjerregaard, M.D.; Jurlander, J.; Klausen, P.; Borregaard, N.; Cowland, J.B. The in Vivo Profile of Transcription Factors during Neutrophil Differentiation in Human Bone Marrow. *Blood* **2003**, *101*, 4322–4332. [CrossRef]
216. Johansen, L.M.; Iwama, A.; Lodie, T.A.; Sasaki, K.; Felsher, D.W.; Golub, T.R.; Tenen, D.G. C-Myc Is a Critical Target for C/EBPα in Granulopoiesis. *Mol. Cell. Biol.* **2001**, *21*, 3789–3806. [CrossRef] [PubMed]
217. Dahl, R.; Walsh, J.C.; Lancki, D.; Laslo, P.; Iyer, S.R.; Singh, H.; Simon, M.C. Regulation of Macrophage and Neutrophil Cell Fates by the PU.1:C/EBPα Ratio and Granulocyte Colony-Stimulating Factor. *Nat. Immunol.* **2003**, *4*, 1029–1036. [CrossRef]
218. Radomska, H.S.; Bassères, D.S.; Zheng, R.; Zhang, P.; Dayaram, T.; Yamamoto, Y.; Sternberg, D.W.; Lokker, N.; Giese, N.A.; Bohlander, S.K.; et al. Block of C/EBPα Function by Phosphorylation in Acute Myeloid Leukemia with FLT3 Activating Mutations. *J. Exp. Med.* **2006**, *203*, 371–381. [CrossRef]
219. Feinberg, M.W.; Wara, A.K.; Cao, Z.; Lebedeva, M.A.; Rosenbauer, F.; Iwasaki, H.; Hirai, H.; Katz, J.P.; Haspel, R.L.; Gray, S.; et al. The Kruppel-like Factor KLF4 Is a Critical Regulator of Monocyte Differentiation. *EMBO J.* **2007**, *26*, 4138–4148. [CrossRef]
220. Hock, H.; Hamblen, M.J.; Rooke, H.M.; Traver, D.; Bronson, R.T.; Cameron, S.; Orkin, S.H. Intrinsic Requirement for Zinc Finger Transcription Factor Gfi-1 in Neutrophil Differentiation. *Immunity* **2003**, *18*, 109–120. [CrossRef]
221. Laslo, P.; Spooner, C.J.; Warmflash, A.; Lancki, D.W.; Lee, H.-J.; Sciammas, R.; Gantner, B.N.; Dinner, A.R.; Singh, H. Multilineage Transcriptional Priming and Determination of Alternate Hematopoietic Cell Fates. *Cell* **2006**, *126*, 755–766. [CrossRef]
222. Schönheit, J.; Kuhl, C.; Gebhardt, M.L.; Klett, F.F.; Riemke, P.; Scheller, M.; Huang, G.; Naumann, R.; Leutz, A.; Stocking, C.; et al. PU.1 Level-Directed Chromatin Structure Remodeling at the Irf8 Gene Drives Dendritic Cell Commitment. *Cell Rep.* **2013**, *3*, 1617–1628. [CrossRef]
223. Burn, G.L.; Foti, A.; Marsman, G.; Patel, D.F.; Zychlinsky, A. The Neutrophil. *Immunity* **2021**, *54*, 1377–1391. [CrossRef]
224. Rosales, C. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? *Front. Physiol.* **2018**, *9*, 113. [CrossRef] [PubMed]
225. Freifeld, A.G.; Bow, E.J.; Sepkowitz, K.A.; Boeckh, M.J.; Ito, J.I.; Mullen, C.A.; Raad, I.I.; Rolston, K.V.; Young, J.-A.H.; Wingard, J.R.; et al. Clinical Practice Guideline for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer: 2010 Update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2011**, *52*, e56–e93. [CrossRef] [PubMed]
226. Pizzo, P.A. Management of Fever in Patients with Cancer and Treatment-Induced Neutropenia. *N. Engl. J. Med.* **1993**, *328*, 1323–1332. [CrossRef] [PubMed]
227. Delaney, C.; Milano, F.; Cicconi, L.; Othus, M.; Becker, P.S.; Sandhu, V.; Nicoud, I.; Dahlberg, A.; Bernstein, I.D.; Appelbaum, F.R.; et al. Infusion of a Non-HLA-Matched Ex-Vivo Expanded Cord Blood Progenitor Cell Product after Intensive Acute Myeloid Leukaemia Chemotherapy: A Phase 1 Trial. *Lancet Haematol.* **2016**, *3*, e330–e339. [CrossRef] [PubMed]
228. Price, T.H.; Boeckh, M.; Harrison, R.W.; McCullough, J.; Ness, P.M.; Strauss, R.G.; Nichols, W.G.; Hamza, T.H.; Cushing, M.M.; King, K.E.; et al. Efficacy of Transfusion with Granulocytes from G-CSF/Dexamethasone-Treated Donors in Neutropenic Patients with Infection. *Blood* **2015**, *126*, 2153–2161. [CrossRef]
229. Desai, P.M.; Brown, J.; Gill, S.; Solh, M.M.; Akard, L.P.; Hsu, J.W.; Ustun, C.; Andreadis, C.; Frankfurt, O.; Foran, J.M.; et al. Open-Label Phase II Prospective, Randomized, Controlled Study of Romyelocel-L Myeloid Progenitor Cells to Reduce Infection during Induction Chemotherapy for Acute Myeloid Leukemia. *JCO* **2021**, *39*, 3261–3272. [CrossRef]
230. De Bruyn, C.; Delforge, A.; Bernier, M.; Bron, D. Ex Vivo Expansion of Neutrophil Precursor Cells from Fresh and Cryopreserved Cord Blood Cells. *Cytotherapy* **2003**, *5*, 87–98. [CrossRef]
231. Hino, M.; Suzuki, K.; Yamane, T.; Sakai, N.; Kubota, H.; Koh, K.R.; Ohta, K.; Hato, F.; Kitagawa, S.; Tatsumi, N. Ex Vivo Expansion of Mature Human Neutrophils with Normal Functions from Purified Peripheral Blood $CD34^+$ Haematopoietic Progenitor Cells. *Br. J. Haematol.* **2000**, *109*, 314–321. [CrossRef]
232. Jie, Z.; Zhang, Y.; Wang, C.; Shen, B.; Guan, X.; Ren, Z.; Ding, X.; Dai, W.; Jiang, Y. Large-Scale Ex Vivo Generation of Human Neutrophils from Cord Blood $CD34^+$ Cells. *PLoS ONE* **2017**, *12*, e0180832. [CrossRef]
233. Kuhikar, R.; Khan, N.; Khare, S.P.; Fulzele, A.; Melinkeri, S.; Kale, V.; Limaye, L. Neutrophils Generated in Vitro from Hematopoietic Stem Cells Isolated from Apheresis Samples and Umbilical Cord Blood Form Neutrophil Extracellular Traps. *Stem Cell Res.* **2021**, *50*, 102150. [CrossRef]

234. Lachmann, N.; Ackermann, M.; Frenzel, E.; Liebhaber, S.; Brennig, S.; Happle, C.; Hoffmann, D.; Klimenkova, O.; Lüttge, D.; Buchegger, T.; et al. Large-Scale Hematopoietic Differentiation of Human Induced Pluripotent Stem Cells Provides Granulocytes or Macrophages for Cell Replacement Therapies. *Stem Cell Rep.* **2015**, *4*, 282–296. [CrossRef]
235. Rodak, B.; Fritsma, G.; Keohane, E. *Hematology: Clinical Principals and Applications*; Elsevier Health Sciences: St. Louis, MO, USA, 2013.
236. Egeland, T.; Steen, R.; Quarsten, H.; Gaudernack, G.; Yang, Y.-C.; Thorsby, E. Myeloid Differentiation of Purified CD34$^+$ Cells after Stimulation with Recombinant Human Granulocyte-Monocyte Colony-Stimulating Factor (CSF), Granulocyte-CSF, Monocyte-CSF, and Interleukin-3. *Blood* **1991**, *78*, 3192–3199. [CrossRef] [PubMed]
237. Timmins, N.E.; Palfreyman, E.; Marturana, F.; Dietmair, S.; Luikenga, S.; Lopez, G.; Fung, Y.L.; Minchinton, R.; Nielsen, L.K. Clinical Scale Ex Vivo Manufacture of Neutrophils from Hematopoietic Progenitor Cells. *Biotechnol. Bioeng.* **2009**, *104*, 832–840. [CrossRef] [PubMed]
238. Tura, O.; Barclay, G.R.; Roddie, H.; Davies, J.; Turner, M.L. Optimal Ex Vivo Expansion of Neutrophils from PBSC CD34$^+$ Cells by a Combination of SCF, Flt3-L and G-CSF and Its Inhibition by Further Addition of TPO. *J. Transl. Med.* **2007**, *5*, 53. [CrossRef] [PubMed]
239. Choi, K.-D.; Vodyanik, M.; Slukvin, I.I. The Hematopoietic Differentiation and Production of Mature Myeloid Cells from Human Pluripotent Stem Cells. *Nat. Protoc.* **2011**, *6*, 296–313. [CrossRef]
240. Bapat, A.; Keita, N.; Sharma, S. Pan-Myeloid Differentiation of Human Cord Blood Derived CD34$^+$ Hematopoietic Stem and Progenitor Cells. *J. Vis. Exp.* **2019**. [CrossRef]
241. Hamilton, J.A. Colony-Stimulating Factors in Inflammation and Autoimmunity. *Nat. Rev. Immunol.* **2008**, *8*, 533–544. [CrossRef]
242. Manz, M.G.; Miyamoto, T.; Akashi, K.; Weissman, I.L. Prospective Isolation of Human Clonogenic Common Myeloid Progenitors. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11872–11877. [CrossRef]
243. Blalock, W.L.; Weinstein-Oppenheimer, C.; Chang, F.; Hoyle, P.E.; Wang, X.-Y.; Algate, P.A.; Franklin, R.A.; Oberhaus, S.M.; Steelman, L.S.; McCubrey, J.A. Signal Transduction, Cell Cycle Regulatory, and Anti-Apoptotic Pathways Regulated by IL-3 in Hematopoietic Cells: Possible Sites for Intervention with Anti-Neoplastic Drugs. *Leukemia* **1999**, *13*, 1109–1166. [CrossRef]
244. Hamilton, J.A. GM-CSF-Dependent Inflammatory Pathways. *Front. Immunol.* **2019**, *10*, 2055. [CrossRef] [PubMed]
245. Hercus, T.R.; Dhagat, U.; Kan, W.L.T.; Broughton, S.E.; Nero, T.L.; Perugini, M.; Sandow, J.J.; D'Andrea, R.J.; Ekert, P.G.; Hughes, T.; et al. Signalling by the Bc Family of Cytokines. *Cytokine Growth Factor Rev.* **2013**, *24*, 189–201. [CrossRef] [PubMed]
246. Clark, S.C.; Kamen, R. The Human Hematopoietic Colony-Stimulating Factors. *Science* **1987**, *236*, 1229–1237. [CrossRef] [PubMed]
247. Welte, K.; Bonilla, M.A.; Gillio, A.P.; Boone, T.C.; Potter, G.K.; Gabrilove, J.L.; Moore, M.A.; O'Reilly, R.J.; Souza, L.M. Recombinant Human Granulocyte Colony-Stimulating Factor. Effects on Hematopoiesis in Normal and Cyclophosphamide-Treated Primates. *J. Exp. Med.* **1987**, *165*, 941–948. [CrossRef] [PubMed]
248. Sweeney, C.L.; Teng, R.; Wang, H.; Merling, R.K.; Lee, J.; Choi, U.; Koontz, S.; Wright, D.G.; Malech, H.L. Molecular Analysis of Neutrophil Differentiation from Human Induced Pluripotent Stem Cells Delineates the Kinetics of Key Regulators of Hematopoiesis. *Stem Cells* **2016**, *34*, 1513–1526. [CrossRef] [PubMed]
249. Barge, R.M.; de Koning, J.P.; Pouwels, K.; Dong, F.; Löwenberg, B.; Touw, I.P. Tryptophan 650 of Human Granulocyte Colony-Stimulating Factor (G-CSF) Receptor, Implicated in the Activation of JAK2, Is Also Required for G-CSF-Mediated Activation of Signaling Complexes of the P21ras Route. *Blood* **1996**, *87*, 2148–2153. [CrossRef]
250. Corey, S.J.; Burkhardt, A.L.; Bolen, J.B.; Geahlen, R.L.; Tkatch, L.S.; Tweardy, D.J. Granulocyte Colony-Stimulating Factor Receptor Signaling Involves the Formation of a Three-Component Complex with Lyn and Syk Protein-Tyrosine Kinases. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4683–4687. [CrossRef]
251. Dwivedi, P.; Greis, K.D. Granulocyte Colony Stimulating Factor Receptor (G-CSFR) Signaling in Severe Congenital Neutropenia, Chronic Neutrophilic Leukemia and Related Malignancies. *Exp. Hematol.* **2017**, *46*, 9–20. [CrossRef]
252. de Koning, J.P.; Soede-Bobok, A.A.; Ward, A.C.; Schelen, A.M.; Antonissen, C.; van Leeuwen, D.; Löwenberg, B.; Touw, I.P. STAT3-Mediated Differentiation and Survival of Myeloid Cells in Response to Granulocyte Colony-Stimulating Factor: Role for the Cyclin-Dependent Kinase Inhibitor P27Kip1. *Oncogene* **2000**, *19*, 3290–3298. [CrossRef]
253. Grishin, A.; Sinha, S.; Roginskaya, V.; Boyer, M.J.; Gomez-Cambronero, J.; Zuo, S.; Kurosaki, T.; Romero, G.; Corey, S.J. Involvement of Shc and Cbl-PI 3-Kinase in Lyn-Dependent Proliferative Signaling Pathways for G-CSF. *Oncogene* **2000**, *19*, 97–105. [CrossRef]
254. Nicholson, S.E.; Novak, U.; Ziegler, S.F.; Layton, J.E. Distinct Regions of the Granulocyte Colony-Stimulating Factor Receptor Are Required for Tyrosine Phosphorylation of the Signaling Molecules JAK2, Stat3, and P42, P44MAPK. *Blood* **1995**, *86*, 3698–3704. [CrossRef]
255. Ilaria, R.L.; Hawley, R.G.; Van Etten, R.A. Dominant Negative Mutants Implicate STAT5 in Myeloid Cell Proliferation and Neutrophil Differentiation. *Blood* **1999**, *93*, 4154–4166. [CrossRef]
256. Hu, N.; Qiu, Y.; Dong, F. Role of Erk1/2 Signaling in the Regulation of Neutrophil Versus Monocyte Development in Response to G-CSF and M-CSF*. *J. Biol. Chem.* **2015**, *290*, 24561–24573. [CrossRef]
257. Jack, G.D.; Zhang, L.; Friedman, A.D. M-CSF Elevates c-Fos and Phospho-C/EBPalpha(S21) via ERK Whereas G-CSF Stimulates SHP2 Phosphorylation in Marrow Progenitors to Contribute to Myeloid Lineage Specification. *Blood* **2009**, *114*, 2172–2180. [CrossRef] [PubMed]
258. Williamson, E.A.; Williamson, I.K.; Chumakov, A.M.; Friedman, A.D.; Koeffler, H.P. CCAAT/Enhancer Binding Protein ϵ: Changes in Function upon Phosphorylation by P38 MAP Kinase. *Blood* **2005**, *105*, 3841–3847. [CrossRef]

259. Dick, E.P.; Prince, L.R.; Sabroe, I. Ex Vivo-Expanded Bone Marrow CD34$^+$ Derived Neutrophils Have Limited Bactericidal Ability. *Stem Cells* **2008**, *26*, 2552–2563. [CrossRef] [PubMed]
260. Mantovani, A.; Sica, A.; Locati, M. New Vistas on Macrophage Differentiation and Activation. *Eur. J. Immunol.* **2007**, *37*, 14–16. [CrossRef]
261. Clanchy, F.I.L.; Holloway, A.C.; Lari, R.; Cameron, P.U.; Hamilton, J.A. Detection and Properties of the Human Proliferative Monocyte Subpopulation. *J. Leukoc. Biol.* **2006**, *79*, 757–766. [CrossRef]
262. Andreesen, R.; Hennemann, B.; Krause, S.W. Adoptive Immunotherapy of Cancer Using Monocyte-Derived Macrophages: Rationale, Current Status, and Perspectives. *J. Leukoc. Biol.* **1998**, *64*, 419–426. [CrossRef] [PubMed]
263. de Souza, V.C.A.; Pereira, T.A.; Teixeira, V.W.; Carvalho, H.; de Castro, M.C.A.B.; D'assunção, C.G.; de Barros, A.F.; Carvalho, C.L.; de Lorena, V.M.B.; Costa, V.M.A.; et al. Bone Marrow-Derived Monocyte Infusion Improves Hepatic Fibrosis by Decreasing Osteopontin, TGF-B1, IL-13 and Oxidative Stress. *World J. Gastroenterol.* **2017**, *23*, 5146–5157. [CrossRef]
264. Green, D.S.; Ning, F.; Duemler, A.; Myers, T.G.; Trewhitt, K.; Ekwede, I.; McCoy, A.; Houston, N.; Lee, J.; Lipkowitz, S.; et al. Intraperitoneal Monocytes plus IFNs as a Novel Cellular Immunotherapy for Ovarian Cancer: Mechanistic Characterization and Results from a Phase I Clinical Trial. *Clin. Cancer Res.* **2023**, *29*, 349–363. [CrossRef]
265. Brennan, P.N.; MacMillan, M.; Manship, T.; Moroni, F.; Glover, A.; Graham, C.; Semple, S.; Morris, D.M.; Fraser, A.R.; Pass, C.; et al. Study Protocol: A Multicentre, Open-Label, Parallel-Group, Phase 2, Randomised Controlled Trial of Autologous Macrophage Therapy for Liver Cirrhosis (MATCH). *BMJ Open* **2021**, *11*, e053190. [CrossRef] [PubMed]
266. Fraser, A.R.; Pass, C.; Burgoyne, P.; Atkinson, A.; Bailey, L.; Laurie, A.; McGowan, N.W.A.; Hamid, A.; Moore, J.K.; Dwyer, B.J.; et al. Development, Functional Characterization and Validation of Methodology for GMP-Compliant Manufacture of Phagocytic Macrophages: A Novel Cellular Therapeutic for Liver Cirrhosis. *Cytotherapy* **2017**, *19*, 1113–1124. [CrossRef]
267. Chernykh, E.R.; Shevela, E.Y.; Starostina, N.M.; Morozov, S.A.; Davydova, M.N.; Menyaeva, E.V.; Ostanin, A.A. Safety and Therapeutic Potential of M2 Macrophages in Stroke Treatment. *Cell Transpl.* **2016**, *25*, 1461–1471. [CrossRef] [PubMed]
268. Chomarat, P.; Banchereau, J.; Davoust, J.; Karolina Palucka, A. IL-6 Switches the Differentiation of Monocytes from Dendritic Cells to Macrophages. *Nat. Immunol.* **2000**, *1*, 510–514. [CrossRef]
269. Menetrier-Caux, C.; Montmain, G.; Dieu, M.C.; Bain, C.; Favrot, M.C.; Caux, C.; Blay, J.Y. Inhibition of the Differentiation of Dendritic Cells from CD34$^+$ Progenitors by Tumor Cells: Role of Interleukin-6 and Macrophage Colony-Stimulating Factor. *Blood* **1998**, *92*, 4778–4791. [CrossRef] [PubMed]
270. Kamps, A.W.; Hendriks, D.; Smit, J.W.; Vellenga, E. Role of Macrophage Colony-Stimulating Factor in the Differentiation and Expansion of Monocytes and Dendritic Cells from CD34$^+$ Progenitor Cells. *Med. Oncol.* **1999**, *16*, 46–52. [CrossRef]
271. Clanchy, F.I.L.; Hamilton, J.A. The Development of Macrophages from Human CD34$^+$ Haematopoietic Stem Cells in Serum-Free Cultures Is Optimized by IL-3 and SCF. *Cytokine* **2013**, *61*, 33–37. [CrossRef]
272. Stec, M.; Weglarczyk, K.; Baran, J.; Zuba, E.; Mytar, B.; Pryjma, J.; Zembala, M. Expansion and Differentiation of CD14$^+$CD16$^-$ and CD14^{++}CD16$^+$ Human Monocyte Subsets from Cord Blood CD34$^+$ Hematopoietic Progenitors. *J. Leukoc. Biol.* **2007**, *82*, 594–602. [CrossRef] [PubMed]
273. Sconocchia, G.; Fujiwara, H.; Rezvani, K.; Keyvanfar, K.; El Ouriaghli, F.; Grube, M.; Melenhorst, J.; Hensel, N.; Barrett, A.J. G-CSF-Mobilized CD34$^+$ Cells Cultured in Interleukin-2 and Stem Cell Factor Generate a Phenotypically Novel Monocyte. *J. Leukoc. Biol.* **2004**, *76*, 1214–1219. [CrossRef] [PubMed]
274. Miyauchi, M.; Ito, Y.; Nakahara, F.; Hino, T.; Nakamura, F.; Iwasaki, Y.; Kawagoshi, T.; Koya, J.; Yoshimi, A.; Arai, S.; et al. Efficient Production of Human Neutrophils from IPSCs That Prevent Murine Lethal Infection with Immune Cell Recruitment. *Blood* **2021**, *138*, 2555–2569. [CrossRef] [PubMed]
275. Duweb, A.; Gaiser, A.-K.; Stiltz, I.; El Gaafary, M.; Simmet, T.; Syrovets, T. The SC Cell Line as an in Vitro Model of Human Monocytes. *J. Leukoc. Biol.* **2022**, *112*, 659–668. [CrossRef] [PubMed]
276. Rey-Giraud, F.; Hafner, M.; Ries, C.H. In Vitro Generation of Monocyte-Derived Macrophages under Serum-Free Conditions Improves Their Tumor Promoting Functions. *PLoS ONE* **2012**, *7*, e42656. [CrossRef] [PubMed]
277. Delneste, Y.; Charbonnier, P.; Herbault, N.; Magistrelli, G.; Caron, G.; Bonnefoy, J.-Y.; Jeannin, P. Interferon-γ Switches Monocyte Differentiation from Dendritic Cells to Macrophages. *Blood* **2003**, *101*, 143–150. [CrossRef] [PubMed]
278. Way, K.J.; Dinh, H.; Keene, M.R.; White, K.E.; Clanchy, F.I.L.; Lusby, P.; Roiniotis, J.; Cook, A.D.; Cassady, A.I.; Curtis, D.J.; et al. The Generation and Properties of Human Macrophage Populations from Hemopoietic Stem Cells. *J. Leukoc. Biol.* **2009**, *85*, 766–778. [CrossRef]
279. Becker, S.; Warren, M.K.; Haskill, S. Colony-Stimulating Factor-Induced Monocyte Survival and Differentiation into Macrophages in Serum-Free Cultures. *J. Immunol.* **1987**, *139*, 3703–3709. [CrossRef]
280. Mossadegh-Keller, N.; Sarrazin, S.; Kandalla, P.K.; Espinosa, L.; Stanley, E.R.; Nutt, S.L.; Moore, J.; Sieweke, M.H. M-CSF Instructs Myeloid Lineage Fate in Single Haematopoietic Stem Cells. *Nature* **2013**, *497*, 239–243. [CrossRef]
281. Pixley, F.J.; Stanley, E.R. CSF-1 Regulation of the Wandering Macrophage: Complexity in Action. *Trends Cell Biol.* **2004**, *14*, 628–638. [CrossRef]
282. Borzillo, G.V.; Ashmun, R.A.; Sherr, C.J. Macrophage Lineage Switching of Murine Early Pre-B Lymphoid Cells Expressing Transduced Fms Genes. *Mol. Cell Biol.* **1990**, *10*, 2703–2714. [CrossRef]

283. Montano Almendras, C.P.; Thudium, C.S.; Löfvall, H.; Moscatelli, I.; Schambach, A.; Henriksen, K.; Richter, J. Forced Expression of Human Macrophage Colony-Stimulating Factor in CD34$^+$ Cells Promotes Monocyte Differentiation in Vitro and in Vivo but Blunts Osteoclastogenesis in Vitro. *Eur. J. Haematol.* **2017**, *98*, 517–526. [CrossRef]
284. Husson, H.; Mograbi, B.; Schmid-Antomarchi, H.; Fischer, S.; Rossi, B. CSF-1 Stimulation Induces the Formation of a Multiprotein Complex Including CSF-1 Receptor, c-Cbl, PI 3-Kinase, Crk-II and Grb2. *Oncogene* **1997**, *14*, 2331–2338. [CrossRef] [PubMed]
285. Kanagasundaram, V.; Jaworowski, A.; Hamilton, J.A. Association between Phosphatidylinositol-3 Kinase, Cbl and Other Tyrosine Phosphorylated Proteins in Colony-Stimulating Factor-1-Stimulated Macrophages. *Biochem. J.* **1996**, *320*, 69–77. [CrossRef] [PubMed]
286. Sampaio, N.G.; Yu, W.; Cox, D.; Wyckoff, J.; Condeelis, J.; Stanley, E.R.; Pixley, F.J. Phosphorylation of CSF-1R Y721 Mediates Its Association with PI3K to Regulate Macrophage Motility and Enhancement of Tumor Cell Invasion. *J. Cell Sci.* **2011**, *124*, 2021–2031. [CrossRef]
287. Stanley, E.R.; Chitu, V. CSF-1 Receptor Signaling in Myeloid Cells. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, a021857. [CrossRef] [PubMed]
288. Chang, M.; Hamilton, J.A.; Scholz, G.M.; Masendycz, P.; Macaulay, S.L.; Elsegood, C.L. Phosphatidylinostitol-3 Kinase and Phospholipase C Enhance CSF-1-Dependent Macrophage Survival by Controlling Glucose Uptake. *Cell. Signal.* **2009**, *21*, 1361–1369. [CrossRef] [PubMed]
289. Takeshita, S.; Faccio, R.; Chappel, J.; Zheng, L.; Feng, X.; Weber, J.D.; Teitelbaum, S.L.; Ross, F.P. C-Fms Tyrosine 559 Is a Major Mediator of M-CSF-Induced Proliferation of Primary Macrophages *. *J. Biol. Chem.* **2007**, *282*, 18980–18990. [CrossRef]
290. Yu, W.; Chen, J.; Xiong, Y.; Pixley, F.J.; Yeung, Y.-G.; Stanley, E.R. Macrophage Proliferation Is Regulated through CSF-1 Receptor Tyrosines 544, 559, and 807. *J. Biol. Chem.* **2012**, *287*, 13694–13704. [CrossRef]
291. Bourgin-Hierle, C.; Gobert-Gosse, S.; Thérier, J.; Grasset, M.-F.; Mouchiroud, G. Src-Family Kinases Play an Essential Role in Differentiation Signaling Downstream of Macrophage Colony-Stimulating Factor Receptors Mediating Persistent Phosphorylation of Phospholipase C-Gamma2 and MAP Kinases ERK1 and ERK2. *Leukemia* **2008**, *22*, 161–169. [CrossRef]
292. Gobert Gosse, S.; Bourgin, C.; Liu, W.Q.; Garbay, C.; Mouchiroud, G. M-CSF Stimulated Differentiation Requires Persistent MEK Activity and MAPK Phosphorylation Independent of Grb2-Sos Association and Phosphatidylinositol 3-Kinase Activity. *Cell. Signal.* **2005**, *17*, 1352–1362. [CrossRef]
293. Jackson, T.R.; Ling, R.E.; Roy, A. The Origin of B-Cells: Human Fetal B Cell Development and Implications for the Pathogenesis of Childhood Acute Lymphoblastic Leukemia. *Front. Immunol.* **2021**, *12*, 637975. [CrossRef]
294. Hystad, M.E.; Myklebust, J.H.; Bø, T.H.; Sivertsen, E.A.; Rian, E.; Forfang, L.; Munthe, E.; Rosenwald, A.; Chiorazzi, M.; Jonassen, I.; et al. Characterization of Early Stages of Human B Cell Development by Gene Expression Profiling1. *J. Immunol.* **2007**, *179*, 3662–3671. [CrossRef] [PubMed]
295. Lin, Y.C.; Jhunjhunwala, S.; Benner, C.; Heinz, S.; Welinder, E.; Mansson, R.; Sigvardsson, M.; Hagman, J.; Espinoza, C.A.; Dutkowski, J.; et al. A Global Network of Transcription Factors, Involving E2A, EBF1 and Foxo1, That Orchestrates B Cell Fate. *Nat. Immunol.* **2010**, *11*, 635–643. [CrossRef] [PubMed]
296. Borghesi, L.; Aites, J.; Nelson, S.; Lefterov, P.; James, P.; Gerstein, R. E47 Is Required for V(D)J Recombinase Activity in Common Lymphoid Progenitors. *J. Exp. Med.* **2005**, *202*, 1669–1677. [CrossRef] [PubMed]
297. Cobaleda, C.; Schebesta, A.; Delogu, A.; Busslinger, M. Pax5: The Guardian of B Cell Identity and Function. *Nat. Immunol.* **2007**, *8*, 463–470. [CrossRef] [PubMed]
298. Lemoine, F.M.; Dedhar, S.; Lima, G.M.; Eaves, C.J. Transformation-Associated Alterations in Interactions Between Pre-B Cells and Fibronectin. *Blood* **1990**, *76*, 2311–2320. [CrossRef] [PubMed]
299. Freedman, A.S.; Rhynhart, K.; Nojima, Y.; Svahn, J.; Eliseo, L.; Benjamin, C.D.; Morimoto, C.; Vivier, E. Stimulation of Protein Tyrosine Phosphorylation in Human B Cells after Ligation of the Beta 1 Integrin VLA-4. *J. Immunol.* **1993**, *150*, 1645–1652. [CrossRef] [PubMed]
300. Tse, K.W.K.; Dang-Lawson, M.; Lee, R.L.; Vong, D.; Bulic, A.; Buckbinder, L.; Gold, M.R. B Cell Receptor-Induced Phosphorylation of Pyk2 and Focal Adhesion Kinase Involves Integrins and the Rap GTPases and Is Required for B Cell Spreading*. *J. Biol. Chem.* **2009**, *284*, 22865–22877. [CrossRef]
301. Kraus, H.; Kaiser, S.; Aumann, K.; Bönelt, P.; Salzer, U.; Vestweber, D.; Erlacher, M.; Kunze, M.; Burger, M.; Pieper, K.; et al. A Feeder-Free Differentiation System Identifies Autonomously Proliferating B Cell Precursors in Human Bone Marrow. *J. Immunol.* **2014**, *192*, 1044–1054. [CrossRef]
302. Namen, A.E.; Lupton, S.; Hjerrild, K.; Wignall, J.; Mochizuki, D.Y.; Schmierer, A.; Mosley, B.; March, C.J.; Urdal, D.; Gillis, S.; et al. Stimulation of B-Cell Progenitors by Cloned Murine Interleukin-7. *Nature* **1988**, *333*, 571–573. [CrossRef]
303. Wolf, M.L.; Buckley, J.A.; Goldfarb, A.; Law, C.L.; LeBien, T.W. Development of a Bone Marrow Culture for Maintenance and Growth of Normal Human B Cell Precursors. *J. Immunol.* **1991**, *147*, 3324–3330. [CrossRef]
304. Lee, G.; Namen, A.E.; Gillis, S.; Ellingsworth, L.R.; Kincade, P.W. Normal B Cell Precursors Responsive to Recombinant Murine IL-7 and Inhibition of IL-7 Activity by Transforming Growth Factor-Beta. *J. Immunol.* **1989**, *142*, 3875–3883. [CrossRef] [PubMed]
305. Dittel, B.N.; LeBien, T.W. The Growth Response to IL-7 during Normal Human B Cell Ontogeny Is Restricted to B-Lineage Cells Expressing CD34. *J. Immunol.* **1995**, *154*, 58–67. [CrossRef]

306. Jiang, Q.; Li, W.Q.; Aiello, F.B.; Mazzucchelli, R.; Asefa, B.; Khaled, A.R.; Durum, S.K. Cell Biology of IL-7, a Key Lymphotrophin. *Cytokine Growth Factor Rev.* **2005**, *16*, 513–533. [CrossRef] [PubMed]
307. Dias, S.; Silva, H., Jr.; Cumano, A.; Vieira, P. Interleukin-7 Is Necessary to Maintain the B Cell Potential in Common Lymphoid Progenitors. *J. Exp. Med.* **2005**, *201*, 971–979. [CrossRef] [PubMed]
308. Kikuchi, K.; Lai, A.Y.; Hsu, C.-L.; Kondo, M. IL-7 Receptor Signaling Is Necessary for Stage Transition in Adult B Cell Development through up-Regulation of EBF. *J. Exp. Med.* **2005**, *201*, 1197–1203. [CrossRef]
309. Åhsberg, J.; Tsapogas, P.; Qian, H.; Zetterblad, J.; Zandi, S.; Månsson, R.; Jönsson, J.-I.; Sigvardsson, M. Interleukin-7-Induced Stat-5 Acts in Synergy with Flt-3 Signaling to Stimulate Expansion of Hematopoietic Progenitor Cells. *J. Biol. Chem.* **2010**, *285*, 36275–36284. [CrossRef]
310. Granato, A.; Hayashi, E.A.; Baptista, B.J.A.; Bellio, M.; Nobrega, A. IL-4 Regulates Bim Expression and Promotes B Cell Maturation in Synergy with BAFF Conferring Resistance to Cell Death at Negative Selection Checkpoints. *J. Immunol.* **2014**, *192*, 5761–5775. [CrossRef]
311. Hipp, N.; Symington, H.; Pastoret, C.; Caron, G.; Monvoisin, C.; Tarte, K.; Fest, T.; Delaloy, C. IL-2 Imprints Human Naive B Cell Fate towards Plasma Cell through ERK/ELK1-Mediated BACH2 Repression. *Nat. Commun.* **2017**, *8*, 1443. [CrossRef]
312. Horikawa, K.; Takatsu, K. Interleukin-5 Regulates Genes Involved in B-Cell Terminal Maturation. *Immunology* **2006**, *118*, 497–508. [CrossRef]
313. Heine, G.; Drozdenko, G.; Grün, J.R.; Chang, H.-D.; Radbruch, A.; Worm, M. Autocrine IL-10 Promotes Human B-Cell Differentiation into IgM- or IgG-Secreting Plasmablasts. *Eur. J. Immunol.* **2014**, *44*, 1615–1621. [CrossRef]
314. Ichii, M.; Oritani, K.; Yokota, T.; Nishida, M.; Takahashi, I.; Shirogane, T.; Ezoe, S.; Saitoh, N.; Tanigawa, R.; Kincade, P.W.; et al. Regulation of Human B Lymphopoiesis by the Transforming Growth Factor-β Superfamily in a Newly Established Coculture System Using Human Mesenchymal Stem Cells as a Supportive Microenvironment. *Exp. Hematol.* **2008**, *36*, 587–597. [CrossRef]
315. Ryan, D.H.; Nuccie, B.L.; Abboud, C.N.; Winslow, J.M. Vascular Cell Adhesion Molecule-I and the Integrin VLA-4 Mediate Adhesion of Human B Cell Precursors to Cultured Bone Marrow Adherent Cells. *J. Clin. Investig.* **1991**, *88*, 995–1004. [CrossRef] [PubMed]
316. Ohkawara, J.-I.; Ikebuchi, K.; Fujihara, M.; Sato, N.; Hirayama, F.; Yamaguchi, M.; Mori, K.J.; Sekiguchi, S. Culture System for Extensive Production of $CD19^+IgM^+$ Cells by Human Cord Blood $CD34^+$ Progenitors. *Leukemia* **1998**, *12*, 764–771. [CrossRef] [PubMed]
317. Miller, J.S.; McCullar, V.; Punzel, M.; Lemischka, I.R.; Moore, K.A. Single Adult Human $CD34^+/Lin^-/CD38^-$ Progenitors Give Rise to Natural Killer Cells, B-Lineage Cells, Dendritic Cells, and Myeloid Cells. *Blood* **1999**, *93*, 96–106. [CrossRef] [PubMed]
318. Prieyl, J.A.; LeBien, T.W. Interleukin 7 Independent Development of Human B Cells. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10348–10353. [CrossRef]
319. Long-Term Culture System for Selective Growth of Human B-Cell Progenitors. Available online: https://www.pnas.org/doi/10.1073/pnas.92.5.1570 (accessed on 3 December 2022).
320. Fluckiger, A.-C.; Sanz, E.; Garcia-Lloret, M.; Su, T.; Hao, Q.-L.; Kato, R.; Quan, S.; de la Hera, A.; Crooks, G.M.; Witte, O.N.; et al. In Vitro Reconstitution of Human B-Cell Ontogeny: From $CD34^+$ Multipotent Progenitors to Ig-Secreting Cells. *Blood* **1998**, *92*, 4509–4520. [CrossRef] [PubMed]
321. Scheeren, F.A.; van Lent, A.U.; Nagasawa, M.; Weijer, K.; Spits, H.; Legrand, N.; Blom, B. Thymic Stromal Lymphopoietin Induces Early Human B-Cell Proliferation and Differentiation. *Eur. J. Immunol.* **2010**, *40*, 955–965. [CrossRef] [PubMed]
322. Li, J.; Bhattacharya, S.; Zhou, J.; Phadnis-Moghe, A.S.; Crawford, R.B.; Kaminski, N.E. Aryl Hydrocarbon Receptor Activation Suppresses EBF1 and PAX5 and Impairs Human B Lymphopoiesis. *J. Immunol.* **2017**, *199*, 3504–3515. [CrossRef]
323. Weber, B.N.; Chi, A.W.-S.; Chavez, A.; Yashiro-Ohtani, Y.; Yang, Q.; Shestova, O.; Bhandoola, A. A Critical Role for TCF-1 in T-Lineage Specification and Differentiation. *Nature* **2011**, *476*, 63–68. [CrossRef]
324. Yui, M.A.; Rothenberg, E.V. Developmental Gene Networks: A Triathlon on the Course to T Cell Identity. *Nat. Rev. Immunol.* **2014**, *14*, 529–545. [CrossRef]
325. Ormandy, L.A.; Hillemann, T.; Wedemeyer, H.; Manns, M.P.; Greten, T.F.; Korangy, F. Increased Populations of Regulatory T Cells in Peripheral Blood of Patients with Hepatocellular Carcinoma. *Cancer Res.* **2005**, *65*, 2457–2464. [CrossRef] [PubMed]
326. Themeli, M.; Rivière, I.; Sadelain, M. New Cell Sources for T Cell Engineering and Adoptive Immunotherapy. *Cell Stem Cell* **2015**, *16*, 357–366. [CrossRef] [PubMed]
327. Palomero, T.; Lim, W.K.; Odom, D.T.; Sulis, M.L.; Real, P.J.; Margolin, A.; Barnes, K.C.; O'Neil, J.; Neuberg, D.; Weng, A.P.; et al. NOTCH1 Directly Regulates C-MYC and Activates a Feed-Forward-Loop Transcriptional Network Promoting Leukemic Cell Growth. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 18261–18266. [CrossRef] [PubMed]
328. Deftos, M.L.; Huang, E.; Ojala, E.W.; Forbush, K.A.; Bevan, M.J. Notch1 Signaling Promotes the Maturation of CD4 and CD8 SP Thymocytes. *Immunity* **2000**, *13*, 73–84. [CrossRef]
329. De Obaldia, M.E.; Bell, J.J.; Wang, X.; Harly, C.; Yashiro-Ohtani, Y.; DeLong, J.H.; Zlotoff, D.A.; Sultana, D.A.; Pear, W.S.; Bhandoola, A. T Cell Development Requires Constraint of the Myeloid Regulator C/EBP-α by the Notch Target and Transcriptional Repressor Hes1. *Nat. Immunol.* **2013**, *14*, 1277–1284. [CrossRef] [PubMed]

330. Weng, A.P.; Millholland, J.M.; Yashiro-Ohtani, Y.; Arcangeli, M.L.; Lau, A.; Wai, C.; del Bianco, C.; Rodriguez, C.G.; Sai, H.; Tobias, J.; et al. C-Myc Is an Important Direct Target of Notch1 in T-Cell Acute Lymphoblastic Leukemia/Lymphoma. *Genes Dev.* **2006**, *20*, 2096–2109. [CrossRef] [PubMed]
331. Poellinger, L.; Lendahl, U. Modulating Notch Signaling by Pathway-Intrinsic and Pathway-Extrinsic Mechanisms. *Curr. Opin. Genet. Dev.* **2008**, *18*, 449–454. [CrossRef] [PubMed]
332. Schmitt, T.M.; Zúñiga-Pflücker, J.C. Induction of T Cell Development from Hematopoietic Progenitor Cells by Delta-like-1 In Vitro. *Immunity* **2002**, *17*, 749–756. [CrossRef]
333. La Motte-Mohs, R.N.; Herer, E.; Zúñiga-Pflücker, J.C. Induction of T-Cell Development from Human Cord Blood Hematopoietic Stem Cells by Delta-like 1 in Vitro. *Blood* **2005**, *105*, 1431–1439. [CrossRef] [PubMed]
334. Awong, G.; Herer, E.; Surh, C.D.; Dick, J.E.; La Motte-Mohs, R.N.; Zúñiga-Pflücker, J.C. Characterization in Vitro and Engraftment Potential in Vivo of Human Progenitor T Cells Generated from Hematopoietic Stem Cells. *Blood* **2009**, *114*, 972–982. [CrossRef]
335. Awong, G.; Herer, E.; La Motte-Mohs, R.N.; Zúñiga-Pflücker, J.C. Human CD8 T Cells Generated in Vitro from Hematopoietic Stem Cells Are Functionally Mature. *BMC Immunol.* **2011**, *12*, 22. [CrossRef] [PubMed]
336. Kato, M.; Masuda, K.; Kakugawa, K.; Kawamoto, H.; Mugishima, H.; Katsura, Y. Quantification of Progenitors Capable of Generating T Cells in Human Cord Blood. *Eur. J. Haematol.* **2008**, *80*, 151–159. [CrossRef] [PubMed]
337. Mohtashami, M.; Shah, D.K.; Nakase, H.; Kianizad, K.; Petrie, H.T.; Zúñiga-Pflücker, J.C. Direct Comparison of Dll1- and Dll4-Mediated Notch Activation Levels Shows Differential Lymphomyeloid Lineage Commitment Outcomes. *J. Immunol.* **2010**, *185*, 867–876. [CrossRef]
338. Hozumi, K.; Mailhos, C.; Negishi, N.; Hirano, K.; Yahata, T.; Ando, K.; Zuklys, S.; Holländer, G.A.; Shima, D.T.; Habu, S. Delta-like 4 Is Indispensable in Thymic Environment Specific for T Cell Development. *J. Exp. Med.* **2008**, *205*, 2507–2513. [CrossRef] [PubMed]
339. Plum, J.; De Smedt, M.; Defresne, M.-P.; Leclercq, G.; Vandekerckhove, B. Human $CD34^+$ Fetal Liver Stem Cells Differentiate to T Cells in a Mouse Thymic Microenvironment. *Blood* **1994**, *84*, 1587–1593. [CrossRef]
340. Poznansky, M.C.; Evans, R.H.; Foxall, R.B.; Olszak, I.T.; Piascik, A.H.; Hartman, K.E.; Brander, C.; Meyer, T.H.; Pykett, M.J.; Chabner, K.T.; et al. Efficient Generation of Human T Cells from a Tissue-Engineered Thymic Organoid. *Nat. Biotechnol.* **2000**, *18*, 729–734. [CrossRef]
341. Chung, B.; Montel-Hagen, A.; Ge, S.; Blumberg, G.; Kim, K.; Klein, S.; Zhu, Y.; Parekh, C.; Balamurugan, A.; Yang, O.O.; et al. Engineering the Human Thymic Microenvironment to Support Thymopoiesis In Vivo. *Stem Cells* **2014**, *32*, 2386–2396. [CrossRef]
342. Seet, C.S.; He, C.; Bethune, M.T.; Li, S.; Chick, B.; Gschweng, E.H.; Zhu, Y.; Kim, K.; Kohn, D.B.; Baltimore, D.; et al. Generation of Mature T Cells from Human Hematopoietic Stem and Progenitor Cells in Artificial Thymic Organoids. *Nat. Methods* **2017**, *14*, 521–530. [CrossRef]
343. Ikawa, T.; Kawamoto, H.; Wright, L.Y.T.; Murre, C. Long-Term Cultured E2A-Deficient Hematopoietic Progenitor Cells Are Pluripotent. *Immunity* **2004**, *20*, 349–360. [CrossRef]
344. Gehre, N.; Nusser, A.; von Muenchow, L.; Tussiwand, R.; Engdahl, C.; Capoferri, G.; Bosco, N.; Ceredig, R.; Rolink, A.G. A Stromal Cell Free Culture System Generates Mouse Pro-T Cells That Can Reconstitute T-Cell Compartments in Vivo. *Eur. J. Immunol.* **2015**, *45*, 932–942. [CrossRef]
345. Reimann, C.; Six, E.; Dal-Cortivo, L.; Schiavo, A.; Appourchaux, K.; Lagresle-Peyrou, C.; de Chappedelaine, C.; Ternaux, B.; Coulombel, L.; Beldjord, K.; et al. Human T-Lymphoid Progenitors Generated in a Feeder-Cell-Free Delta-Like-4 Culture System Promote T-Cell Reconstitution in NOD/SCID/$\gamma c^{-/-}$ Mice. *Stem Cells* **2012**, *30*, 1771–1780. [CrossRef] [PubMed]
346. Simons, L.; Ma, K.; de Chappedelaine, C.; Moiranghtem, R.D.; Elkaim, E.; Olivré, J.; Susini, S.; Appourchaux, K.; Reimann, C.; Sadek, H.; et al. Generation of Adult Human T-Cell Progenitors for Immunotherapeutic Applications. *J. Allergy Clin. Immunol.* **2018**, *141*, 1491–1494.e4. [CrossRef] [PubMed]
347. Aoyama, K.; Delaney, C.; Varnum-Finney, B.; Kohn, A.D.; Moon, R.T.; Bernstein, I.D. The Interaction of the Wnt and Notch Pathways Modulates Natural Killer versus T Cell Differentiation. *Stem Cells* **2007**, *25*, 2488–2497. [CrossRef]
348. Huijskens, M.J.A.J.; Walczak, M.; Koller, N.; Briedé, J.J.; Senden-Gijsbers, B.L.M.G.; Schnijderberg, M.C.; Bos, G.M.J.; Germeraad, W.T.V. Technical Advance: Ascorbic Acid Induces Development of Double-Positive T Cells from Human Hematopoietic Stem Cells in the Absence of Stromal Cells. *J. Leukoc. Biol.* **2014**, *96*, 1165–1175. [CrossRef] [PubMed]
349. Shukla, S.; Langley, M.A.; Singh, J.; Edgar, J.M.; Mohtashami, M.; Zúñiga-Pflücker, J.C.; Zandstra, P.W. Progenitor T-Cell Differentiation from Hematopoietic Stem Cells Using Delta-like-4 and VCAM-1. *Nat. Methods* **2017**, *14*, 531–538. [CrossRef]
350. Trotman-Grant, A.C.; Mohtashami, M.; De Sousa Casal, J.; Martinez, E.C.; Lee, D.; Teichman, S.; Brauer, P.M.; Han, J.; Anderson, M.K.; Zúñiga-Pflücker, J.C. DL4-Mbeads Induce T Cell Lineage Differentiation from Stem Cells in a Stromal Cell-Free System. *Nat. Commun.* **2021**, *12*, 5023. [CrossRef]
351. Edgar, J.M.; Michaels, Y.S.; Zandstra, P.W. Multi-Objective Optimization Reveals Time- and Dose-Dependent Inflammatory Cytokine-Mediated Regulation of Human Stem Cell Derived T-Cell Development. *NPJ Regen. Med.* **2022**, *7*, 1–13. [CrossRef]
352. Hernández-López, C.; Varas, A.; Sacedón, R.; Jiménez, E.; Muñoz, J.J.; Zapata, A.G.; Vicente, A. Stromal Cell–Derived Factor 1/CXCR4 Signaling Is Critical for Early Human T-Cell Development. *Blood* **2002**, *99*, 546–554. [CrossRef]
353. Janas, M.L.; Varano, G.; Gudmundsson, K.; Noda, M.; Nagasawa, T.; Turner, M. Thymic Development beyond β-Selection Requires Phosphatidylinositol 3-Kinase Activation by CXCR4. *J. Exp. Med.* **2009**, *207*, 247–261. [CrossRef]

354. Ross, S.H.; Cantrell, D.A. Signaling and Function of Interleukin-2 in T Lymphocytes. *Annu. Rev. Immunol.* **2018**, *36*, 411–433. [CrossRef]
355. Van Acker, H.H.; Anguille, S.; Willemen, Y.; Van den Bergh, J.M.; Berneman, Z.N.; Lion, E.; Smits, E.L.; Van Tendeloo, V.F. Interleukin-15 Enhances the Proliferation, Stimulatory Phenotype, and Antitumor Effector Functions of Human Gamma Delta T Cells. *J. Hematol. Oncol.* **2016**, *9*, 101. [CrossRef] [PubMed]
356. Lefort, N.; Benne, C.; Lelièvre, J.D.; Dorival, C.; Balbo, M.; Sakano, S.; Coulombel, L.; Lévy, Y. Short Exposure to Notch Ligand Delta-4 Is Sufficient to Induce T-Cell Differentiation Program and to Increase the T Cell Potential of Primary Human $CD34^+$ Cells. *Exp. Hematol.* **2006**, *34*, 1720–1729. [CrossRef] [PubMed]